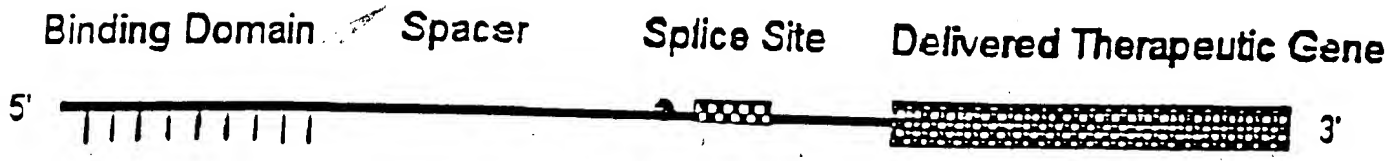
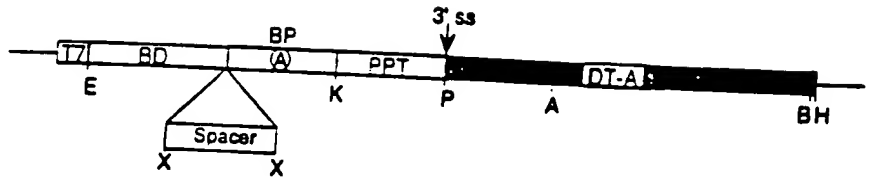
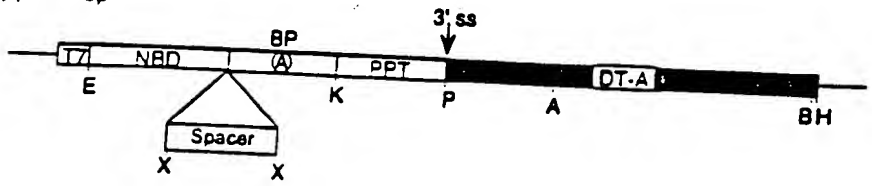
FIGURE 1A


Binding Domain
Spacer
Splice Site
Delivered Therapeutic Gene
5'
3'

(B) (1) pPTM+Sp



(2) pPTM-Sp



(C)

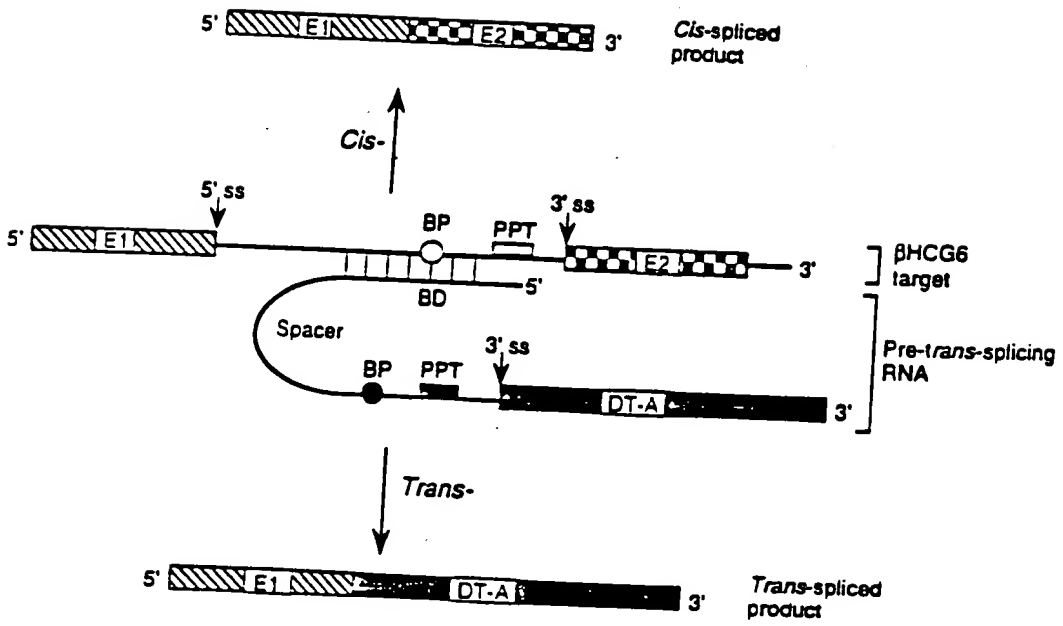
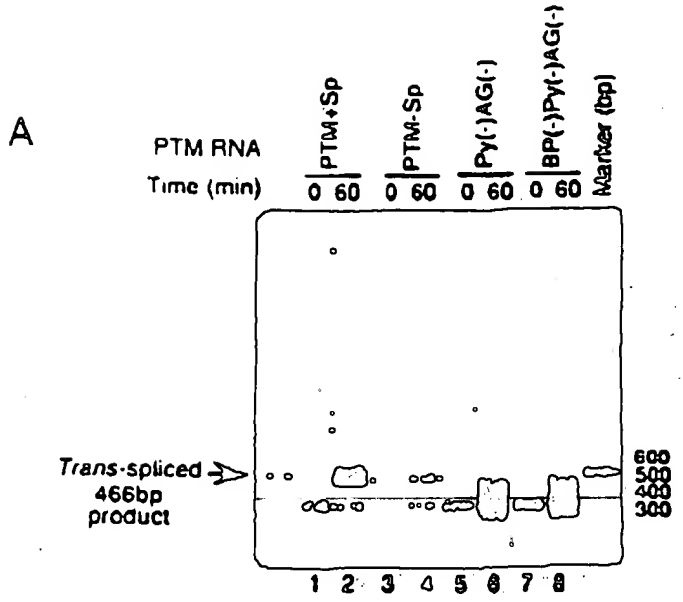
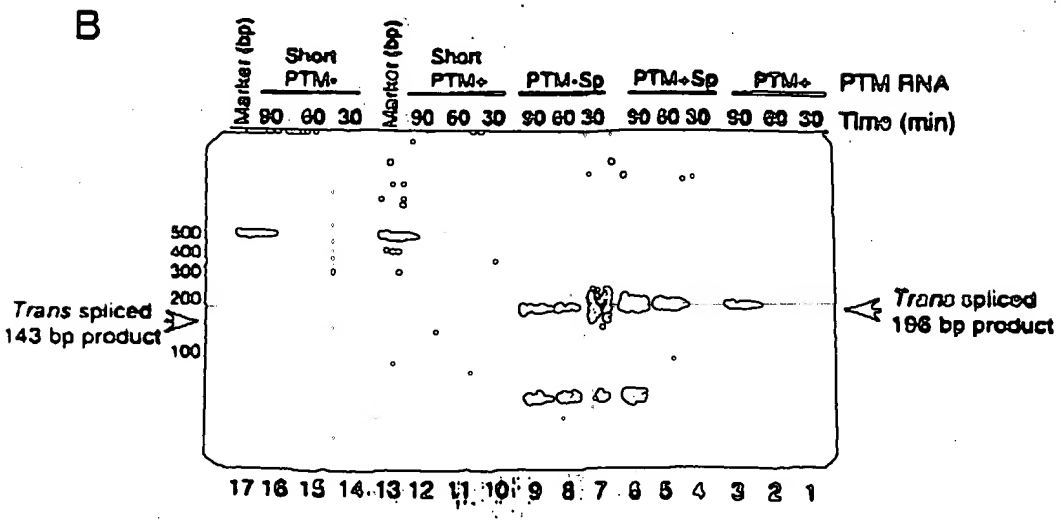
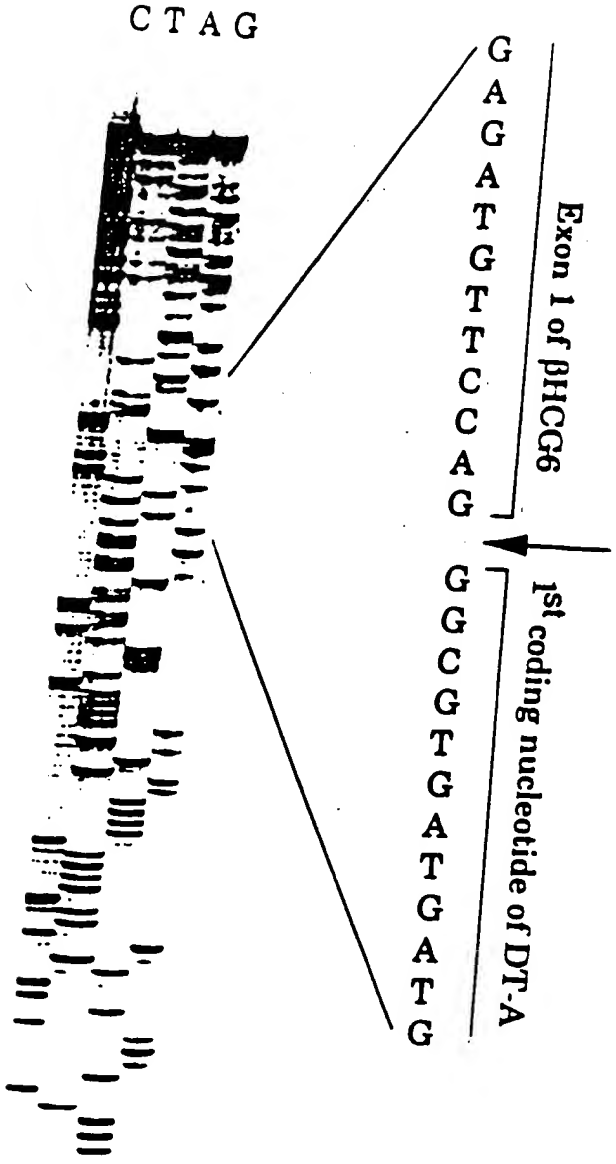


Figure 1B-C

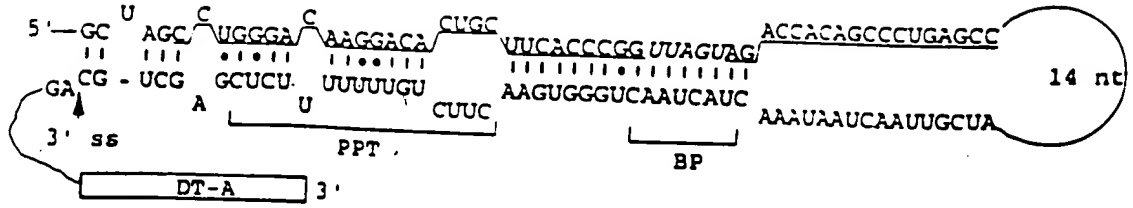

A
PTM RNA
Time (min)
PTM+Sp
PTM-Sp
Py(-)AG(-)
BP(-)Py(-)AG(-)
Marker (bp)
0 60 0 60 0 60 0 60
Trans-spliced
466bp
product
600
500
400
300
1 2 3 4 5 6 7 8


B
Marker (bp)
Short
PTM+
Marker (bp)
Short
PTM+
PTM-Sp
PTM+Sp
PTM+
PTM RNA
90 60 30 90 60 30 90 60 30 90 60 30 90 60 30
Time (min)
500
400
300
200
100
Trans spliced
143 bp product
Trans spliced
196 bp product
17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

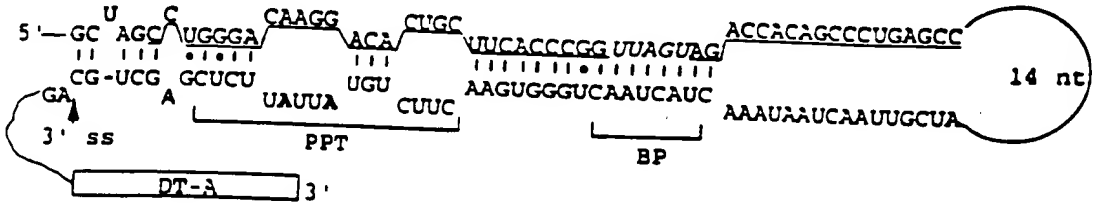

C T A G
G A G A T G T T C C A G
Exon 1 of βHCG6
G G C G T G A T G A T G
1st coding nucleotide of DT-A

(A)

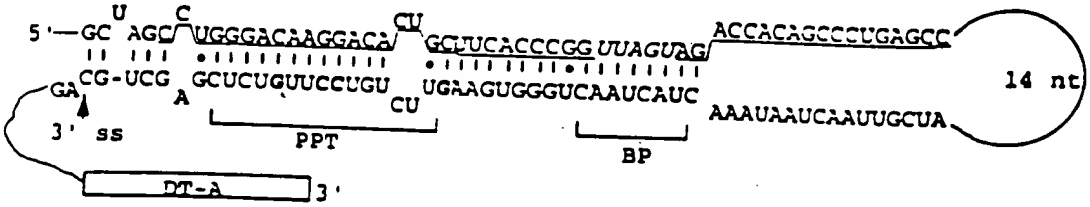
1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:



(B)

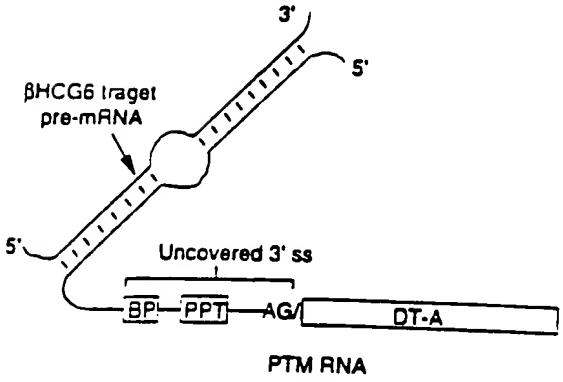


Figure 4A-B

(C)

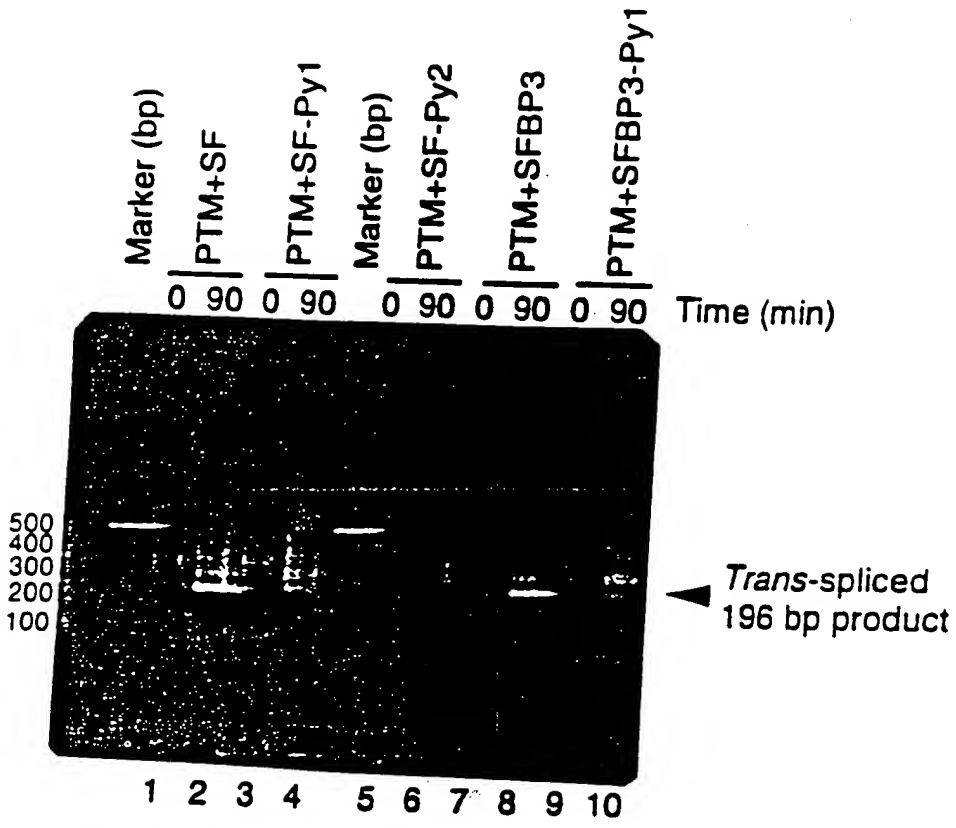

Marker (bp)
PTM+SF
PTM+SF-Py1
Marker (bp)
PTM+SF-Py2
PTM+SFBP3
PTM+SFBP3-Py1
0 90 0 90 0 90 0 90 0 90 0 90
Time (min)
500
400
300
200
100
Trans-spliced
196 bp product
1 2 3 4 5 6 7 8 9 10

Figure 4C

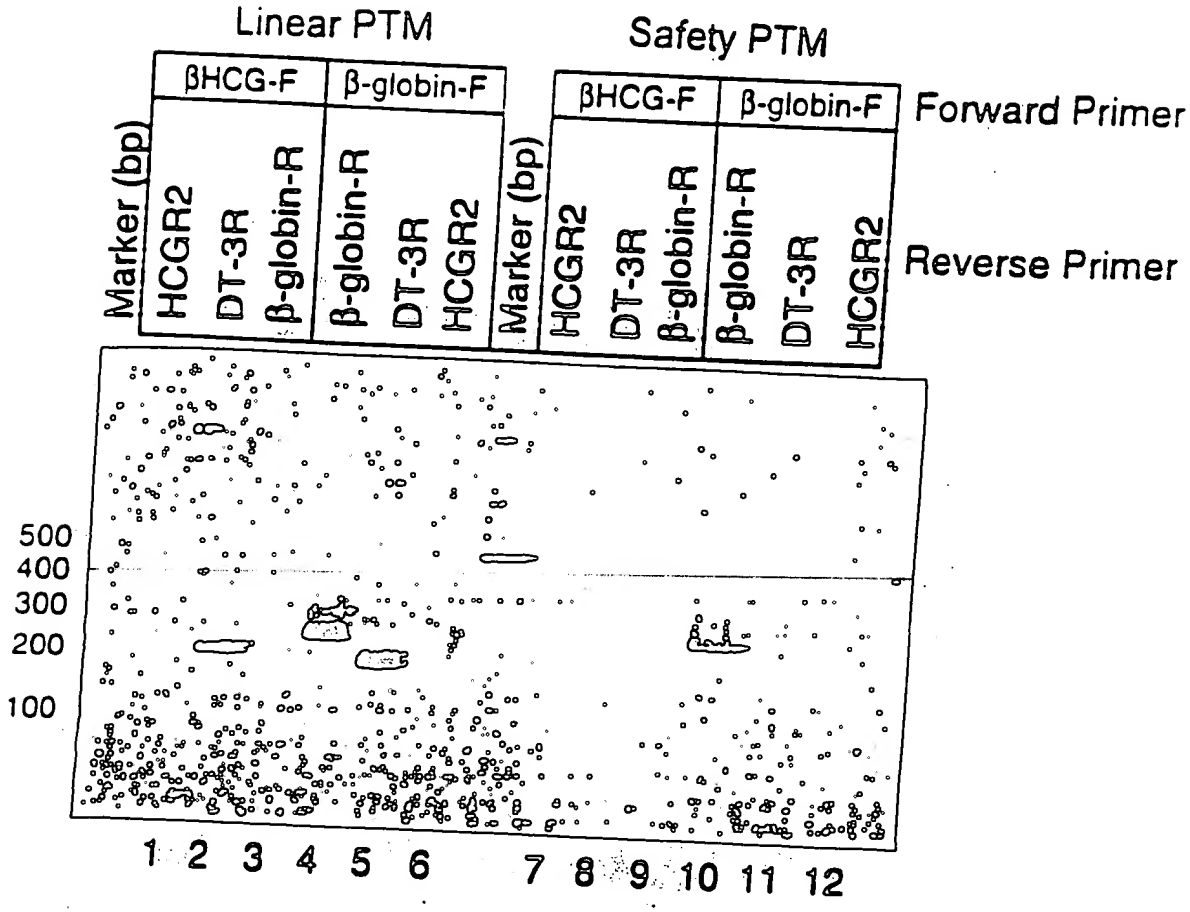

Linear PTM
Safety PTM
βHCG-F
β-globin-F
Forward Primer
Reverse Primer
Marker (bp)
HCGR2
DT-3R
β-globin-R
500
400
300
200
100
1 2 3 4 5 6 7 8 9 10 11 12

Figure 5

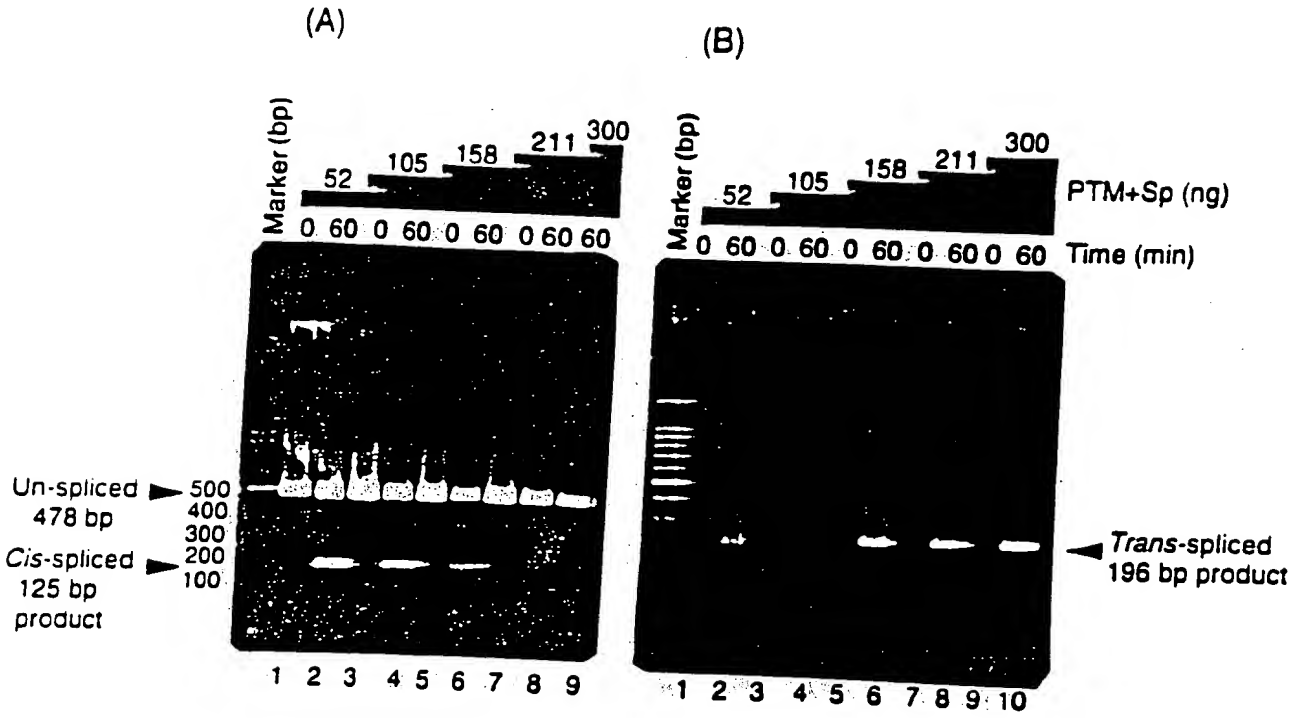
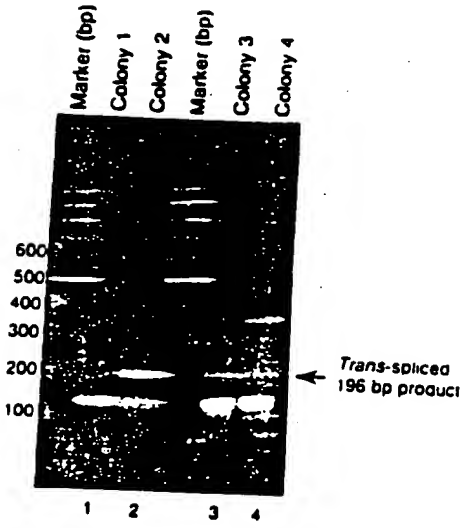

(A)
(B)
Marker (bp)
52
105
158
211
300
0 60 0 60 0 60 0 60 60
PTM+Sp (ng)
Time (min)
Un-spliced
478 bp
Cis-spliced
125 bp
product
500
400
300
200
100
Trans-spliced
196 bp product
1 2 3 4 5 6 7 8 9
1 2 3 4 5 6 7 8 9 10

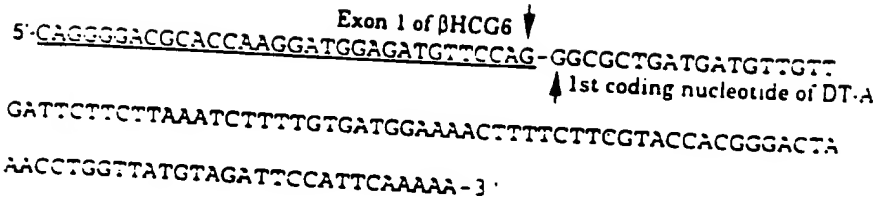
Figure 6

Figure 7

(A)


Marker (bp)
Colony 1
Colony 2
Marker (bp)
Colony 3
Colony 4
600
500
400
300
200
100
Trans-spliced
196 bp product
1
2
3
4

(B)


Exon 1 of βHCG6
5'-CAGGGGACGCACCAAGGATGGAGATGTTCCAG-GGCGCTGATGATGTTGTT
1st coding nucleotide of DT-A
GATTCTTCTTAAATCTTTTGTGATGGAAAACTTTTCTTCGTACCACGGGACTA
AACCTGGTTATGTAGATTCCATTCAAAAA-3'

Double Splicing Pre-therapeutic RNA

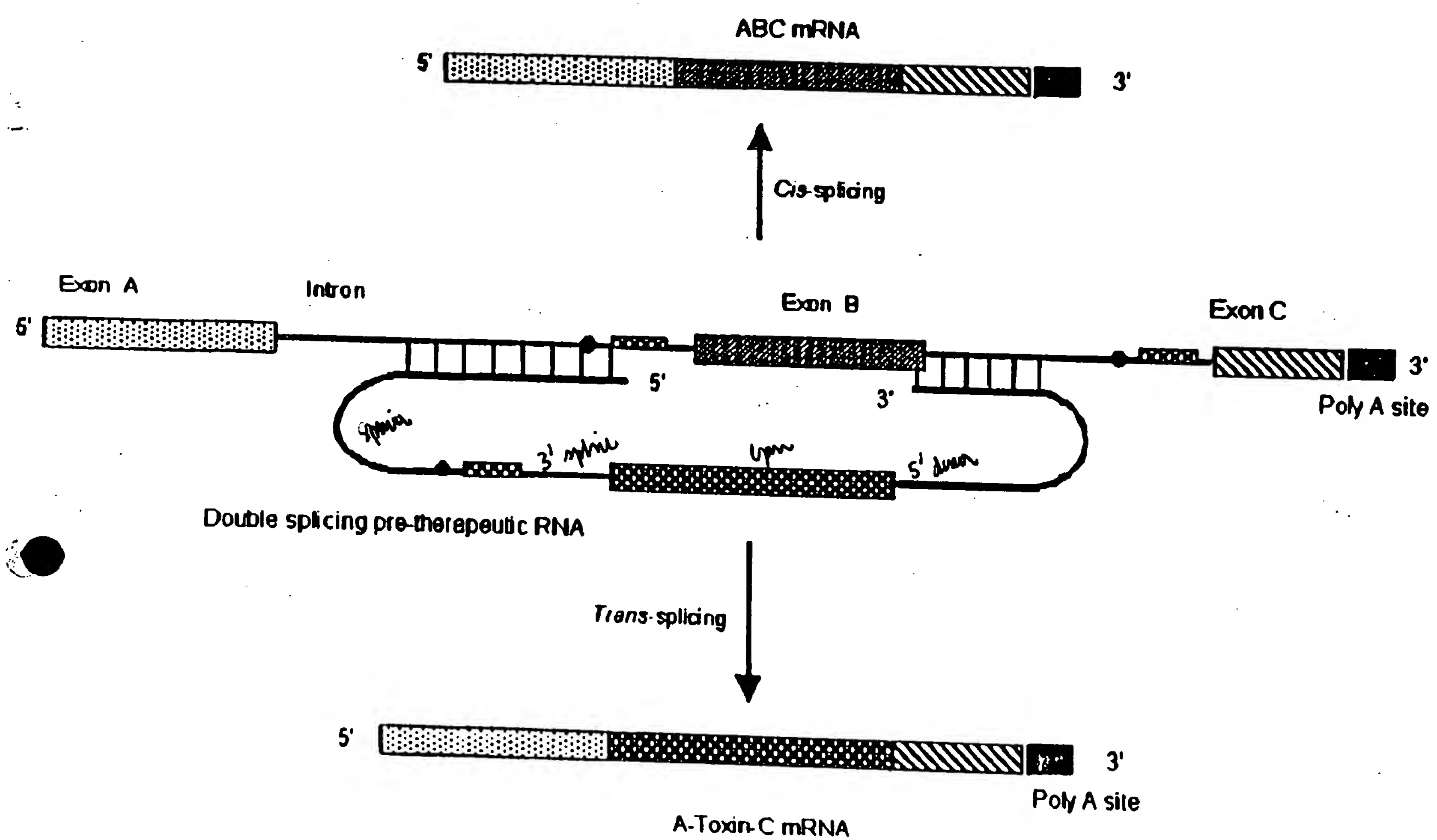
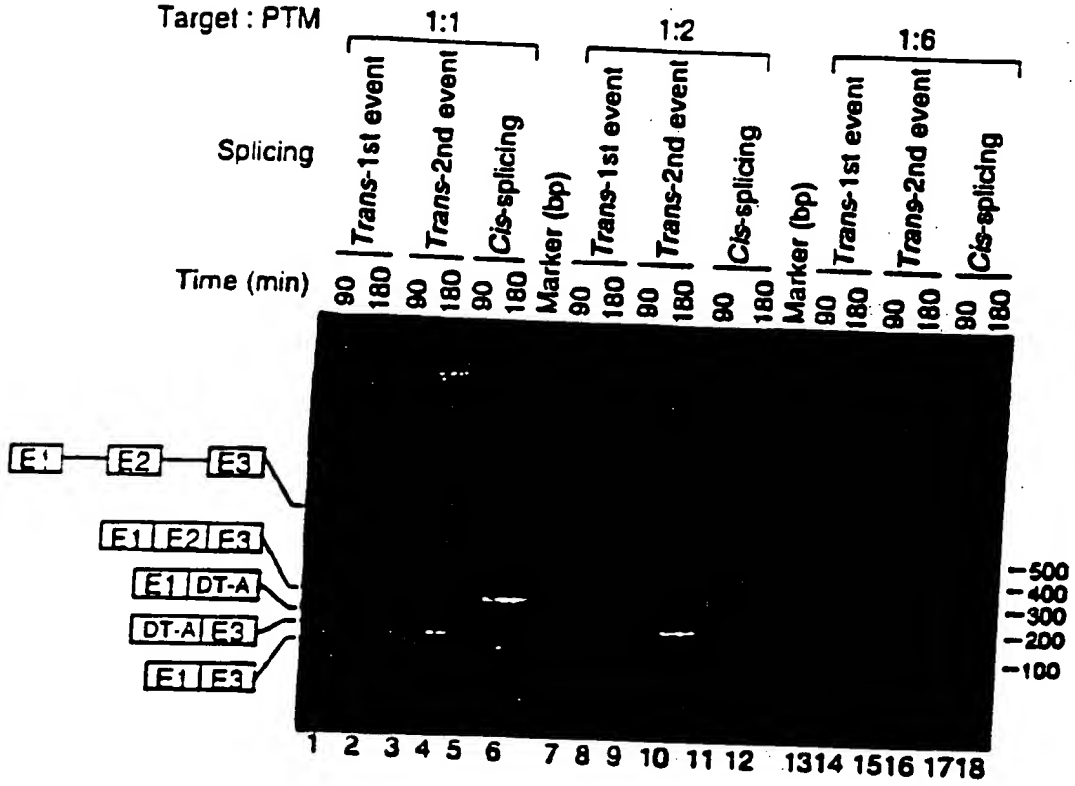


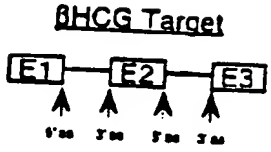
Figure 8A

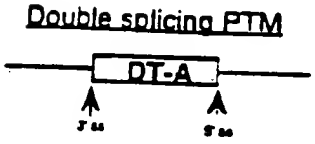
31304B-A
(Sheet 10 of 58)

Selective *Trans*-splicing of a Double Splicing PTM

(3' ss of PTM to 5' ss target and, 5' ss of PTM to 3' ss of target)







Cis-spliced products

E1 E2 E3 = Normal *cis*-splicing (277bp)

E1 E3 = Exon skipping (110bp)

Trans-splicied products

E1 DT-A = 1st event, 196bp. *Trans*-splicing between 5' ss of target & 3' ss of PTM.

DT-A E3 = 2nd event, 161bp. *Trans*-splicing between 3' ss of target & 5' ss of PTM.

Figure 8B

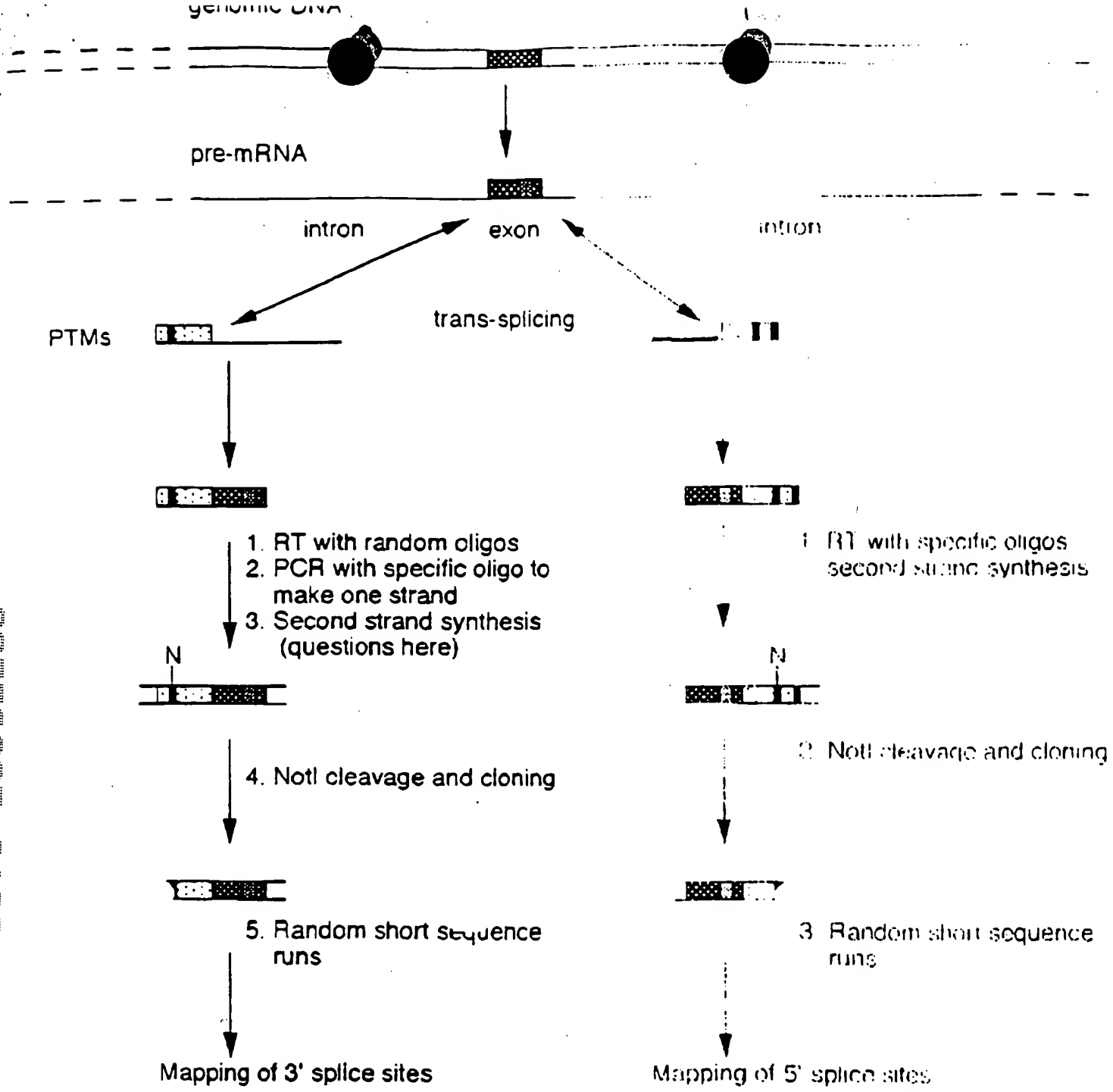

genomic DNA
pre-mRNA
intron
exon
intron
PTMs
trans-splicing
1. RT with random oligos
2. PCR with specific oligo to make one strand
3. Second strand synthesis (questions here)
N
4. NotI cleavage and cloning
5. Random short sequence runs
Mapping of 3' splice sites
1. RT with specific oligos
second strand synthesis
N
2. NotI cleavage and cloning
3. Random short sequence runs
Mapping of 5' splice sites

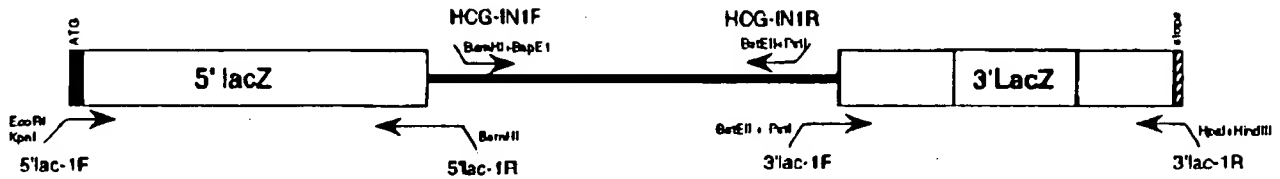
FIGURE 9

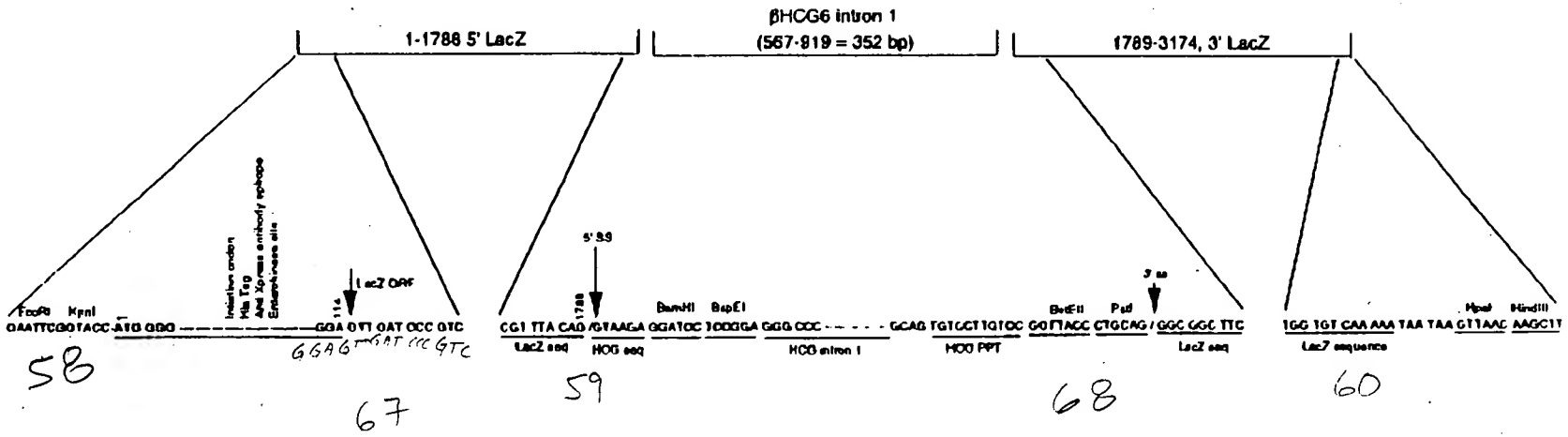
Knock Out

LacZ [redacted] Model Constructs

pc3·1lac-T1

Target 1:





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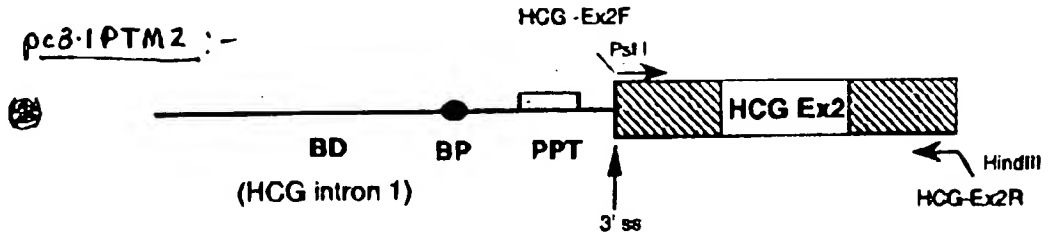
sheet 13 of 58
FIG. 10A

PTMs

pc3·1PTM2 :-



Restoration of β-Gal activity by SMaRT

(Spliceosome Mediated RNA*Trans*-splicing)

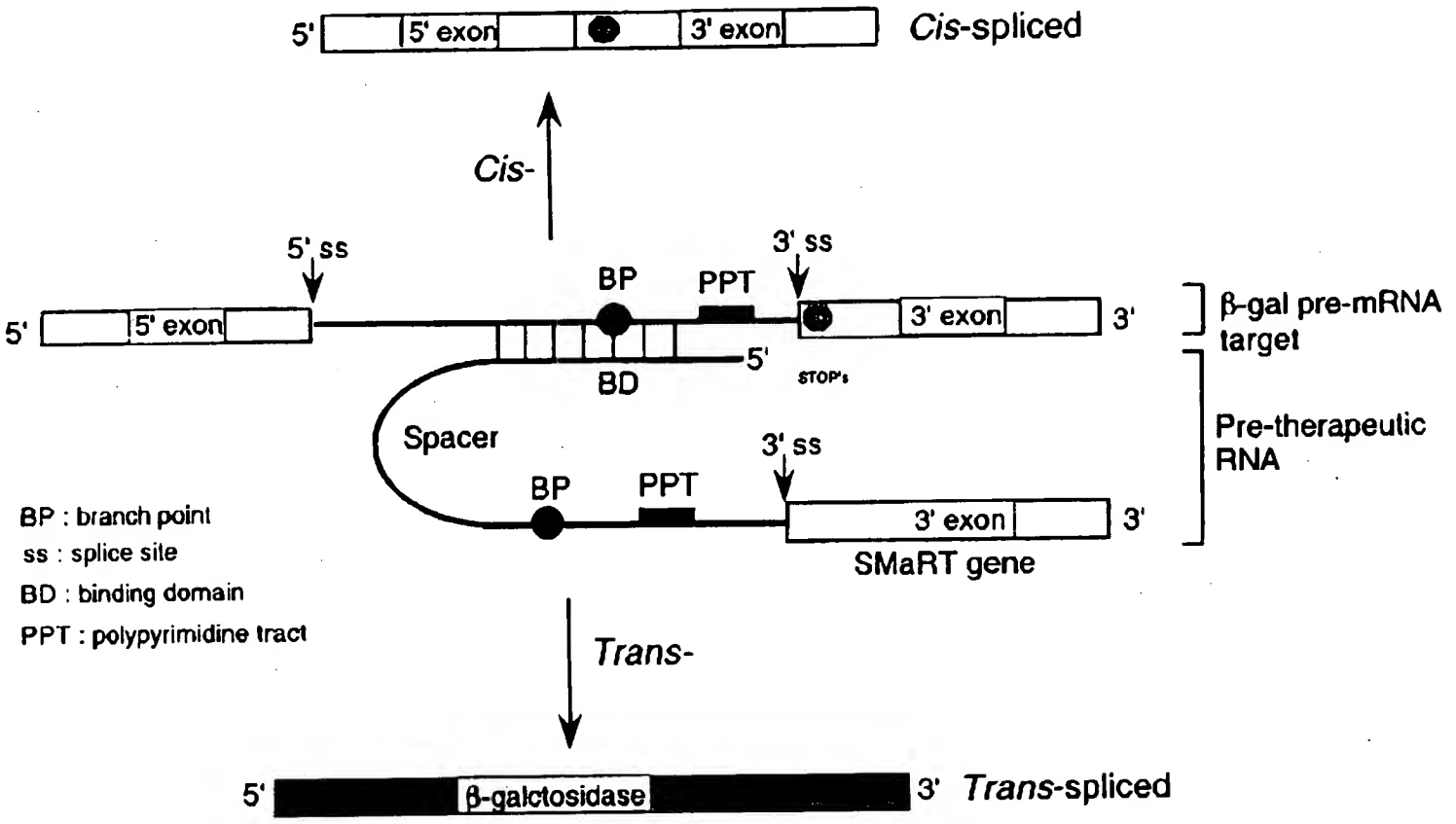


Figure 1D B

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(Amt 14 of 41)

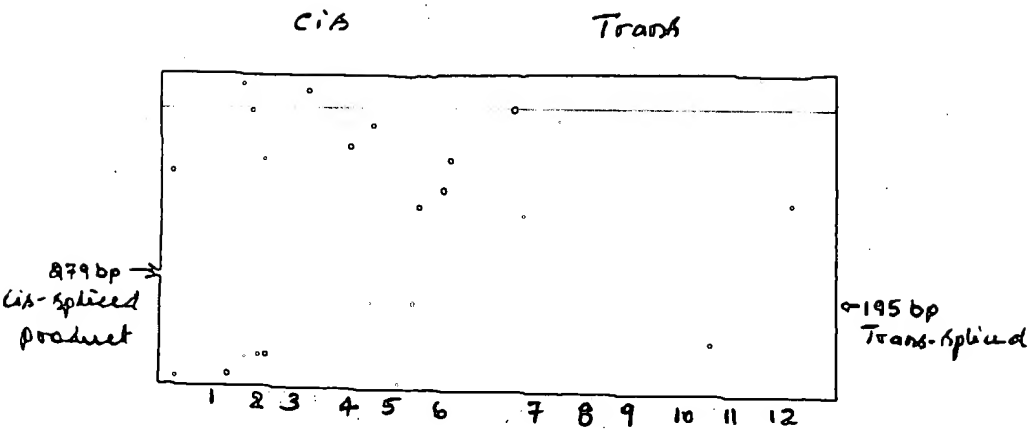
Cis
Trans
879 bp
Cis-spliced
product
195 bp
Trans-Spliced
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 11A

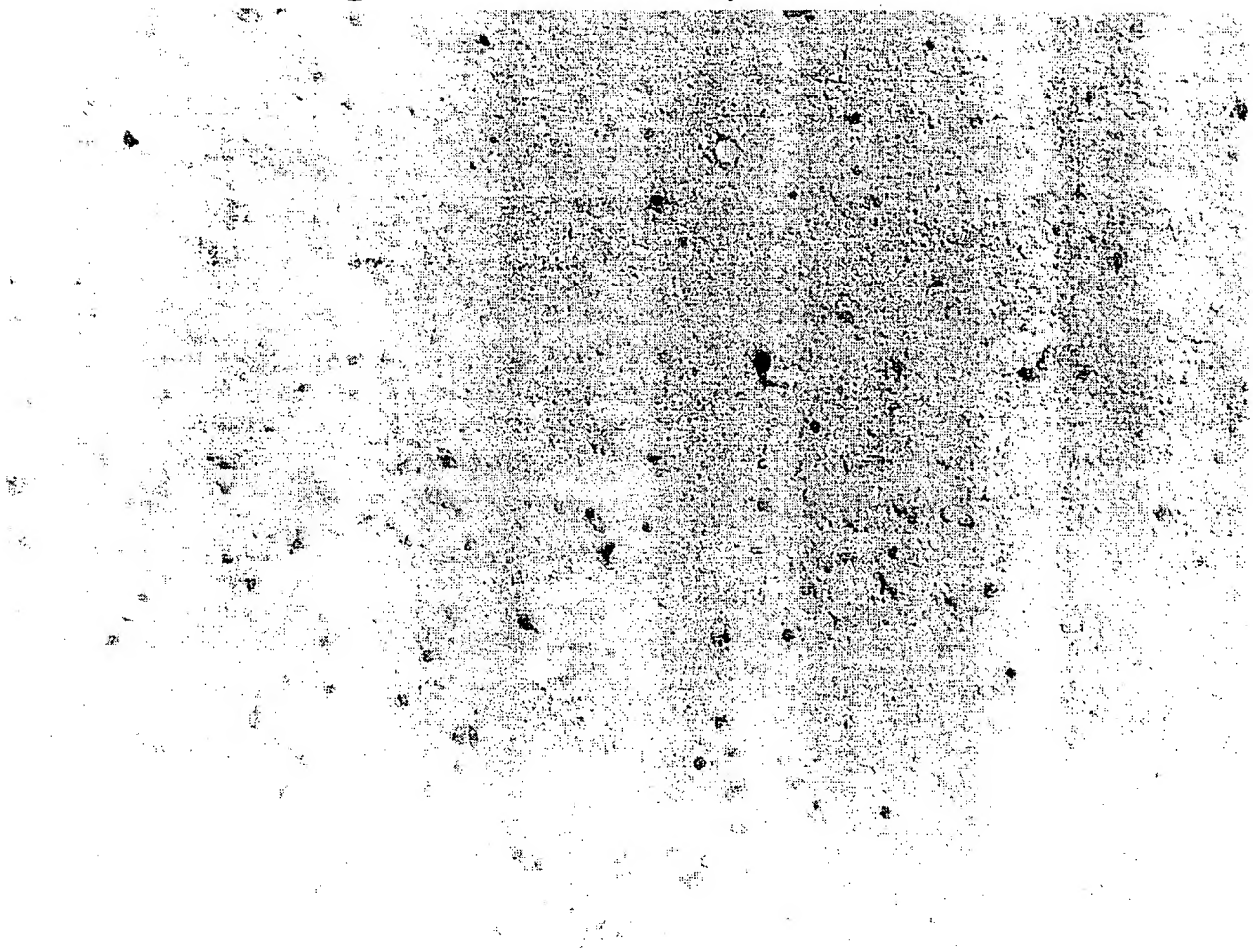

FIGURE IIC

Nucleotide Sequence Demonstrating that *Trans*-splicing is Accurate

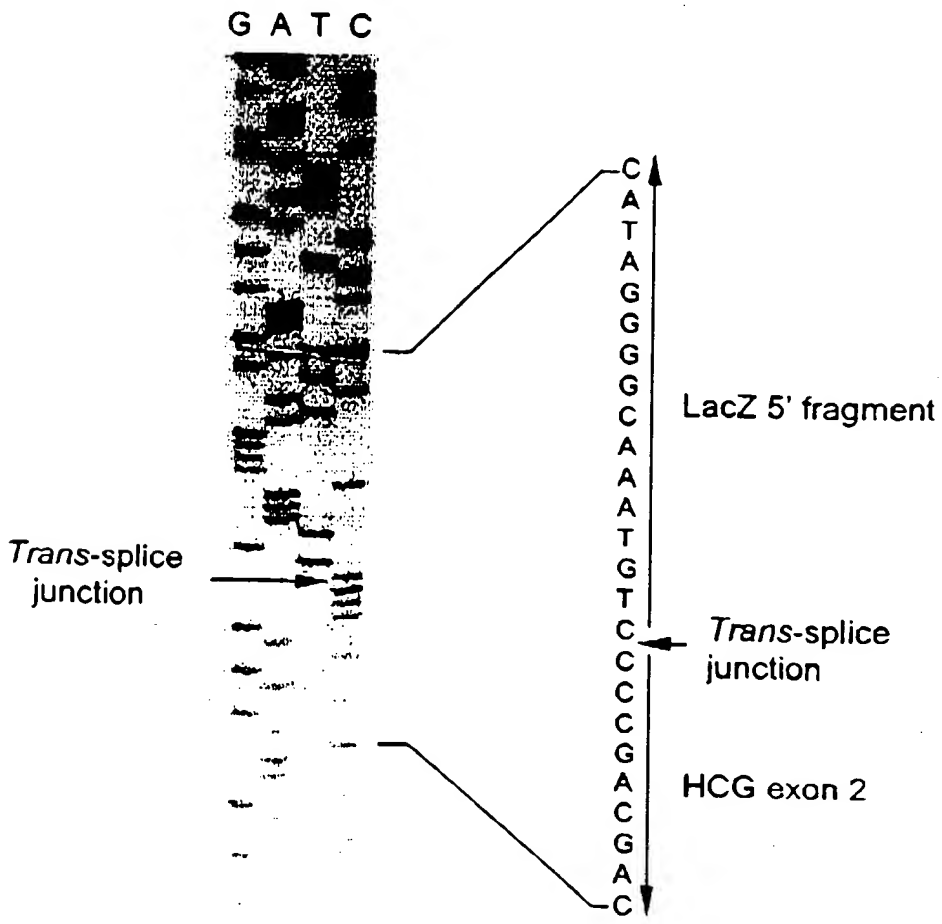

FIGURE 12A

31304-B-A

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(1). Nucleotide sequences of the *cis*-spliced product (285 bp) :

BioLac-TR1
GGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTG

Splice junction
GCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGCGGCTTCGTCTAATAATG

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTT

Lac-TR2
TGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGGCGACCGCACGCCGCATCCAG

(2) Nucleotide sequences of the trans-spliced product (195 bp)

BioLac-TR1
GGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGG

Splice junction
CGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGGCTGCTGCTGTTGCTGCTGCT

HCGR2
GAGCATGGGCGGGACATGGGCATCCAAGGAGCCACTTCGGCCACGGTGCCG

Figure 12 B

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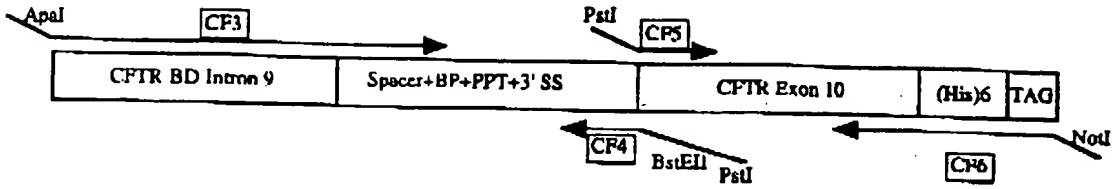
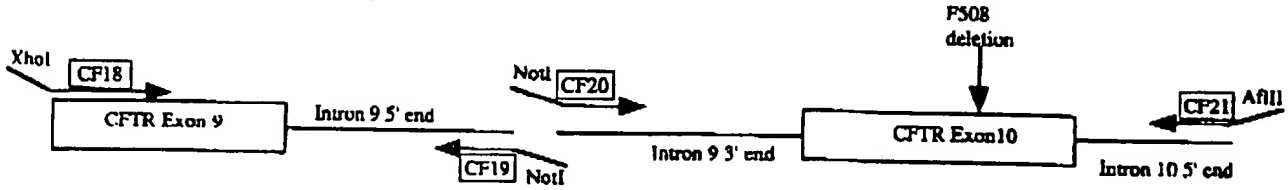
(Shut 19 of 58)

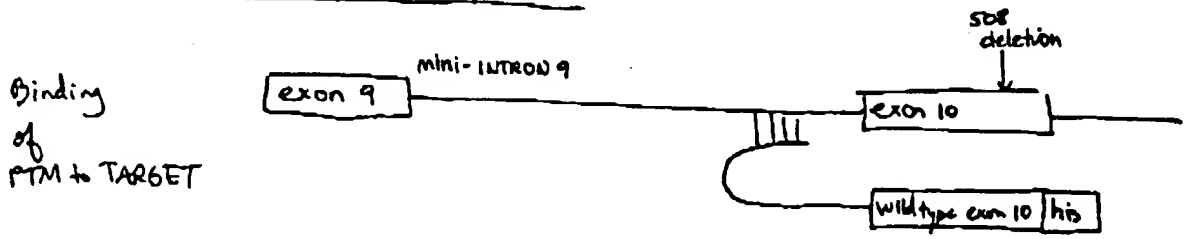
CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target - Construction



TRANS-SPLICING Repair



↓ splicing

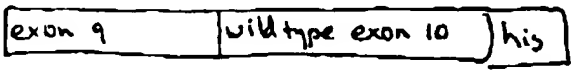


Figure 13

31304-B-A
(Shut 2004.58)

Figure 14

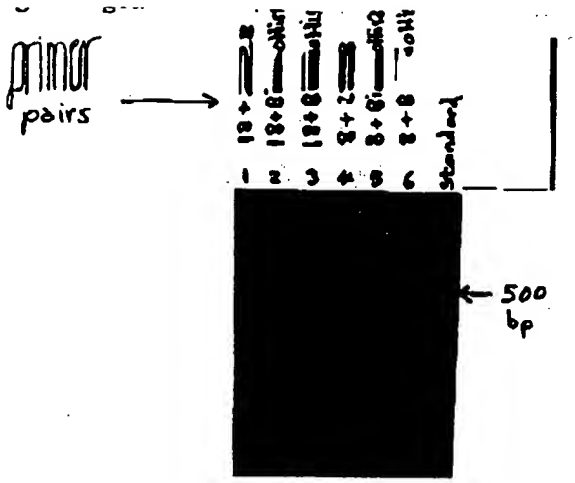


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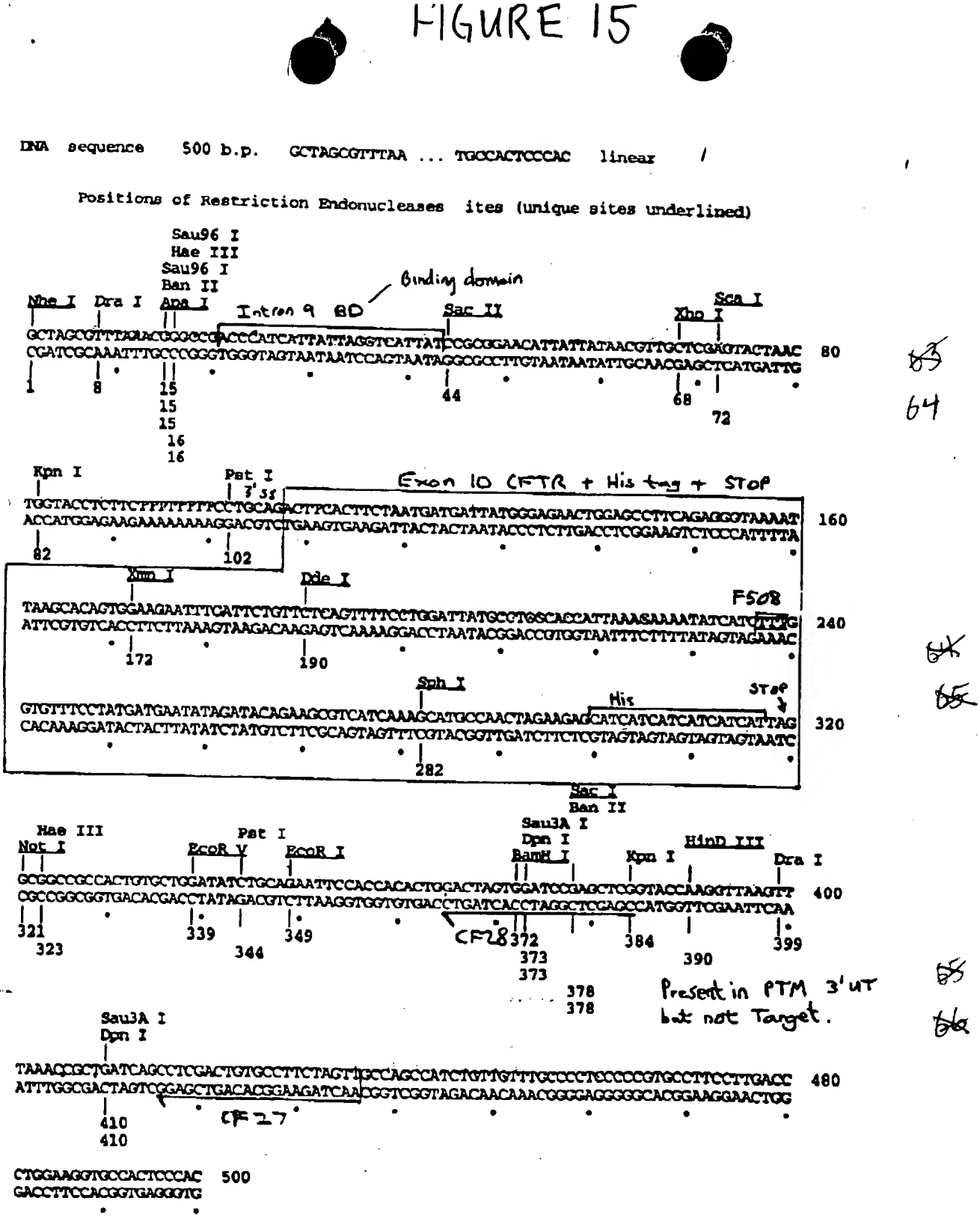
FIGURE 15



Restriction Endonucleases site usage

Acc I	-	EcoR I	1	Nde I	-	Sau96 I	2
Apa I	1	EcoR V	1	Nhe I	1	Sca I	1
ApaL I	-	Hae II	-	Not I	1	Sma I	-
Avr II	-	Hae III	2	PflM I	-	Sph I	1
BamH I	1	HinC II	-	Pst I	2	Spl I	-
Ban II	2	HinD III	1	Pvu I	-	Ssp I	-
Bbe I	-	Hinf I	-	Pvu II	-	Stu I	-

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(Amdt. 22 of 58)

EXPERIMENT 12

Repair of an exogenously supplied CFTR target molecule carrying an F508 deletion in exon 10.

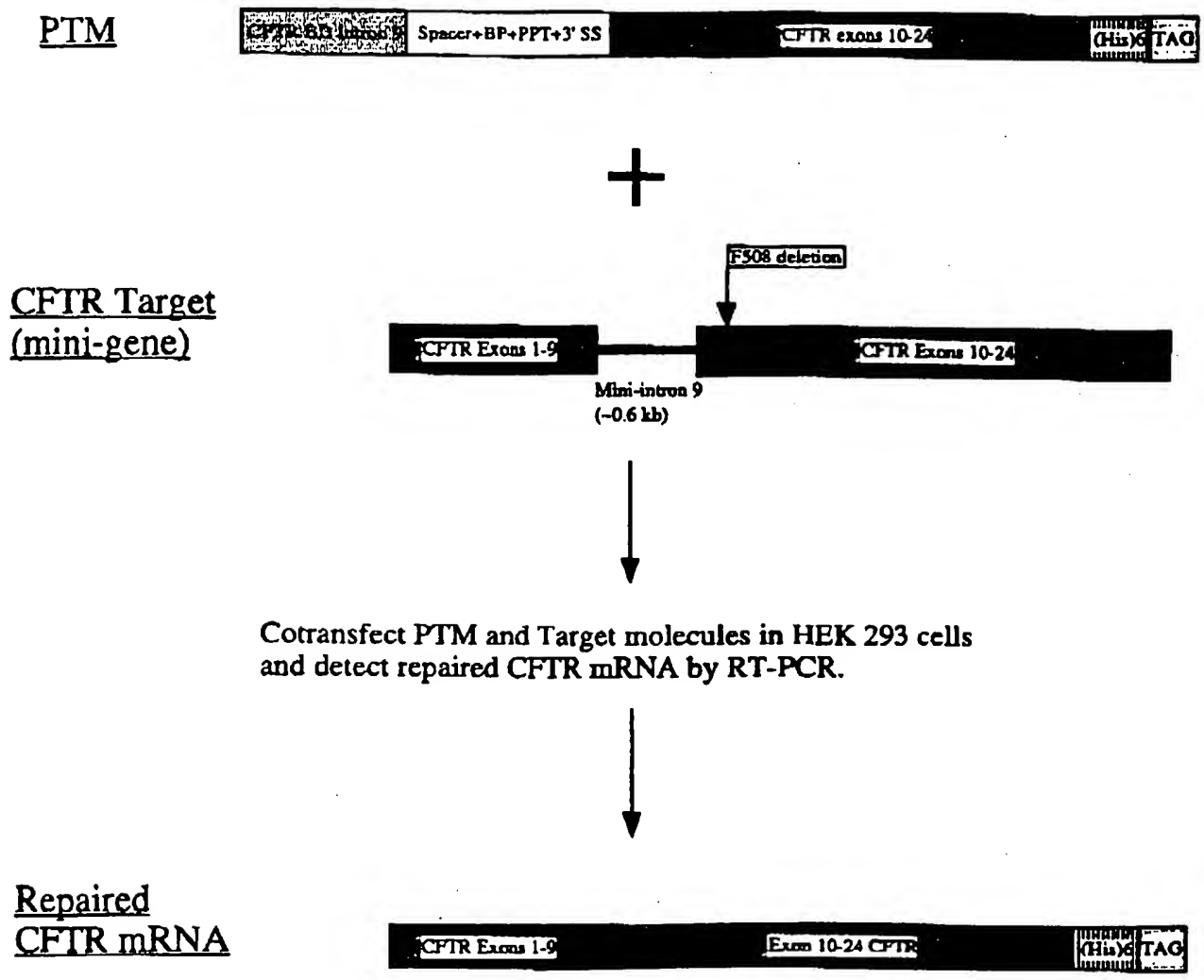

Figure 16

31304-A-B

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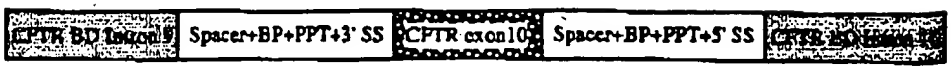
EXPERIMENT 3

Repair of endogenous CFTR transcripts by exon 10 invasion using a double splicing PTM

Double Splicing PTM



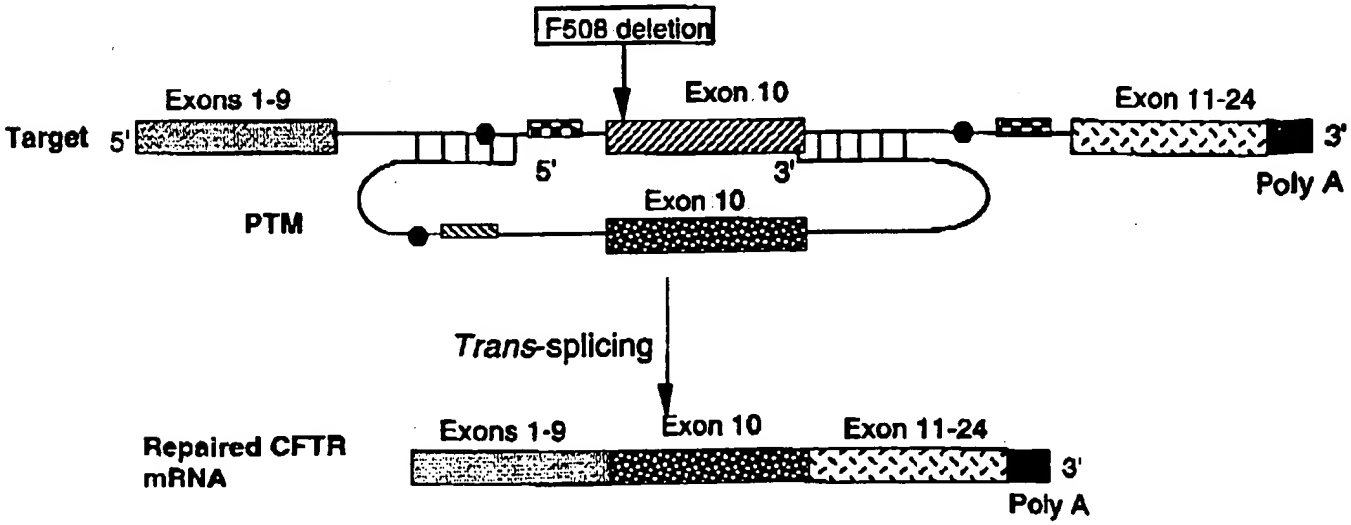


Figure 17

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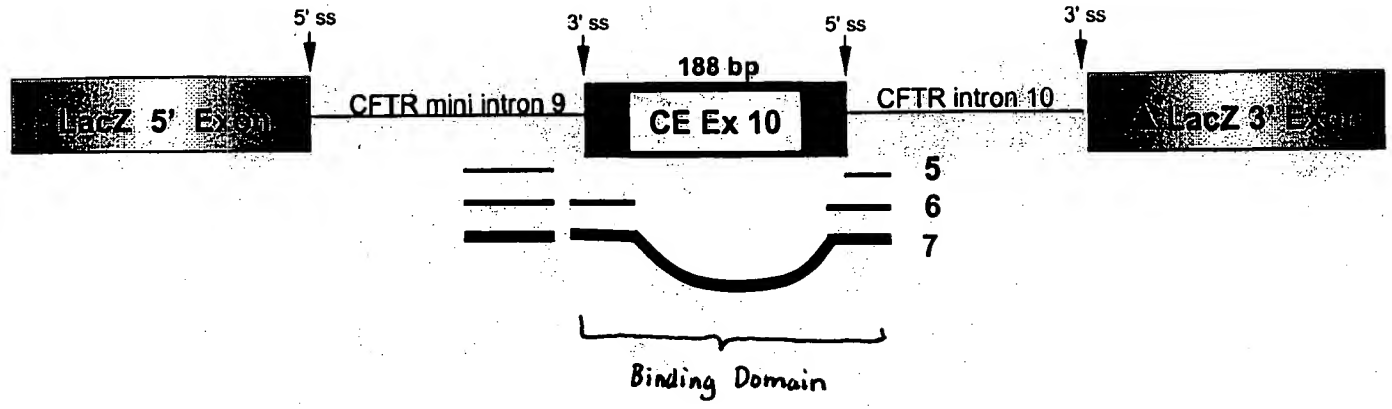
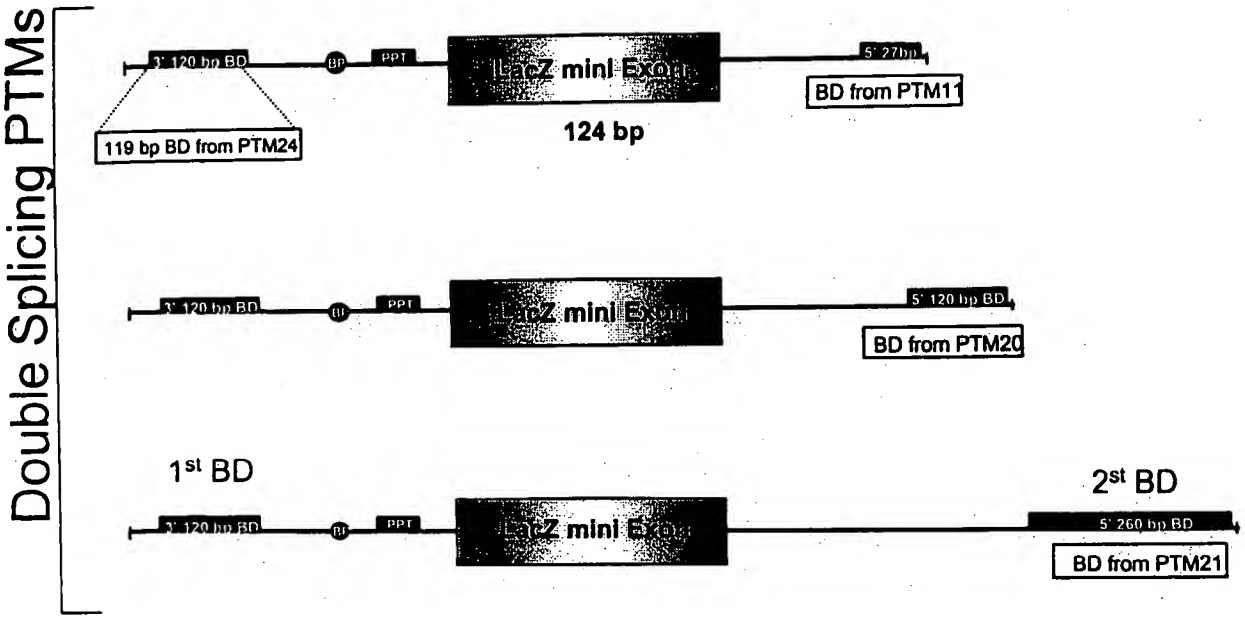
Double *Trans*-splicing Specific Target



Target DSCFT1-6

Figure 18

Double *Trans*-splicing PTMs



DSPTM-5

PTM with 27 bp BD & masks 5' single splice site

DSPTM-6

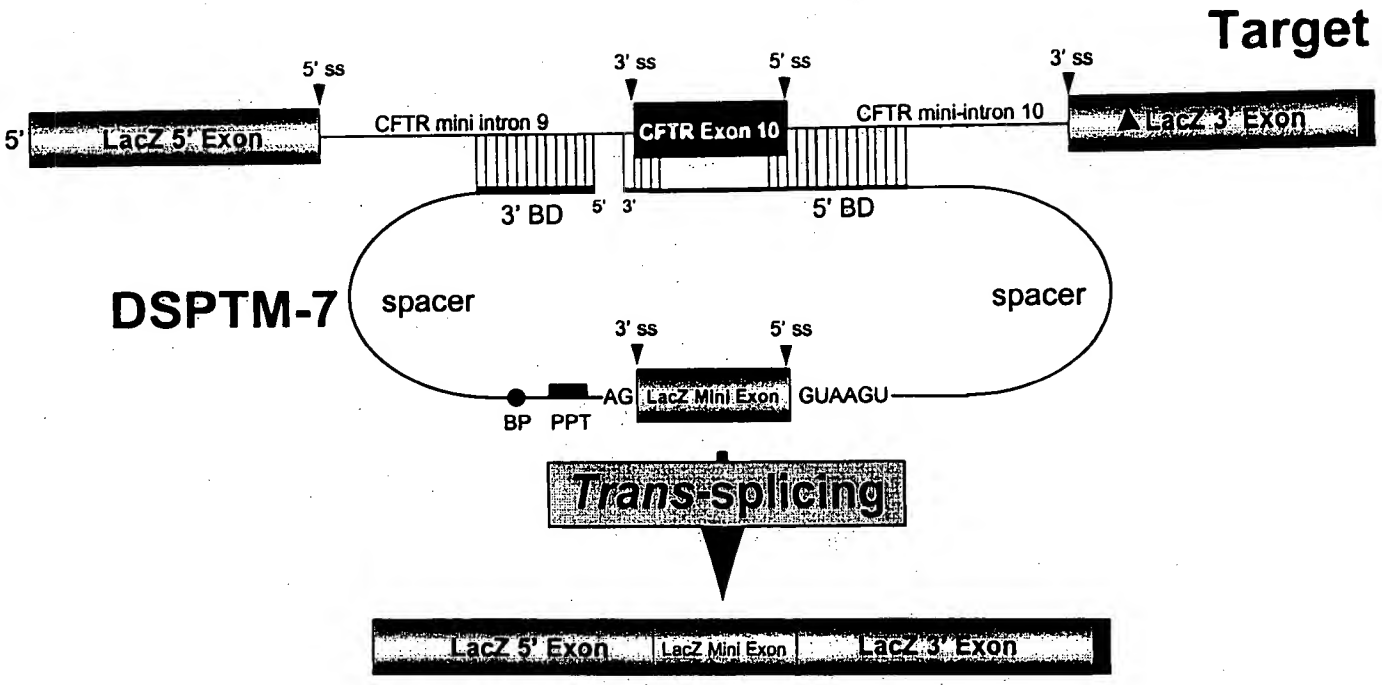
PTM with 120 bp BD & masks both 5' & 3' splice sites

DSPTM-7

PTM with 260 bp BD masking both the ss & the entire CFTR Ex10

Figure 19

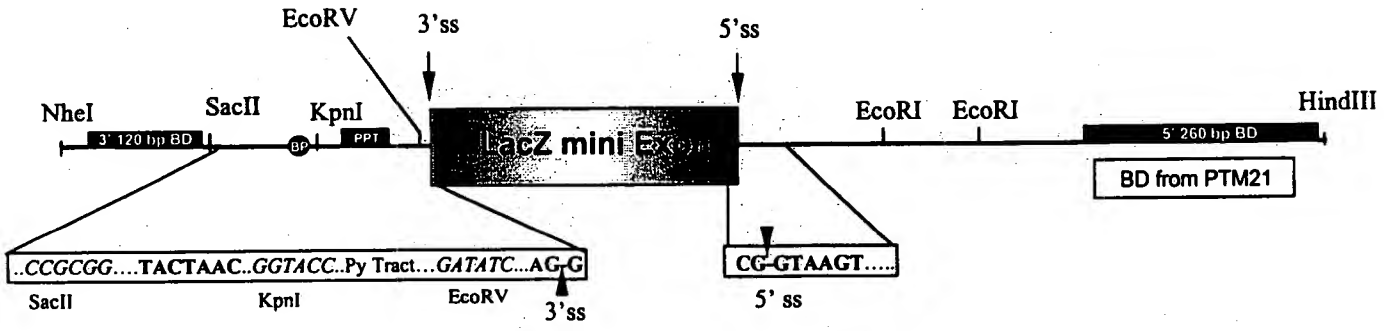
Double *Trans*-splicing β-Gal Model



Repaired LacZ mRNA

Figure 20

Important Structural Elements of DSPTM-7: (Double splicing PTM with all the necessary splice elements i.e. has both 3' and 5' functional splice sites and the binding domains)



(1) 3' BD (120 BP) : GATTCACTTGCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTATTTGTAAAGATTCTATTAACTCATTTGATTC
AAAATATTTAAAATACTTCCTGTTTCATACTCTGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATTATAACGTTGCTCGAA

(3) Branch point, pyrimidine tract and acceptor splice site: TACTAAC T *GGTACC* TCTTCTTTTTTTTTT *GATATC* CTGCAG GGC GGG

3' ss
LacZ mini exon
BP
Kpn I
PPT
EcoRV

(4) 5' donor site and 2nd spacer sequence: TGA ACG *GTAAGT* GTTATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACG

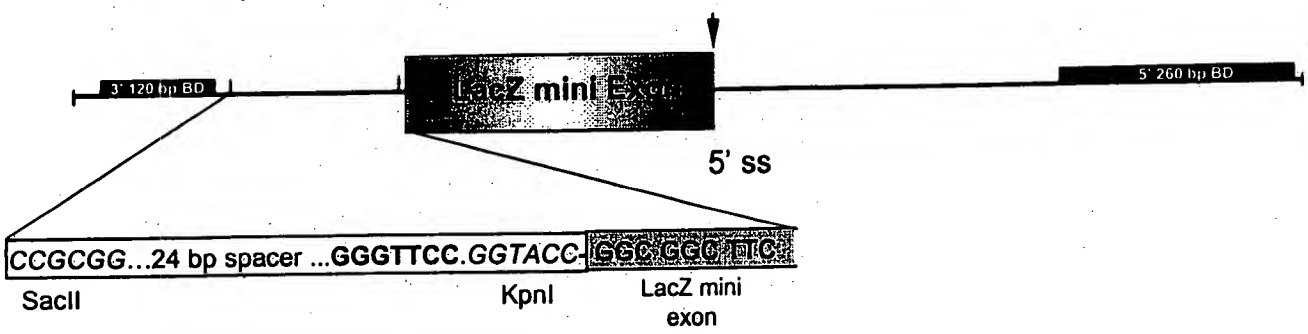
5' ss
LacZ mini exon

CTAAGATCCACCGG

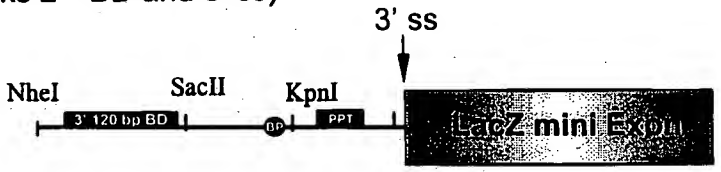
(5) 5' BD (260 BP) : TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTT*GAATTC*ATGCTTTGATGACGCTTCTGTATCTATATTCATCATTGGAA
ACACCAATGATTTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGTGCTTAA
AAAAACCCTCT*GAATTC*TCCATTTCTCCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATTATTAACTCA
TTATCAAATCACGC

Figure 21

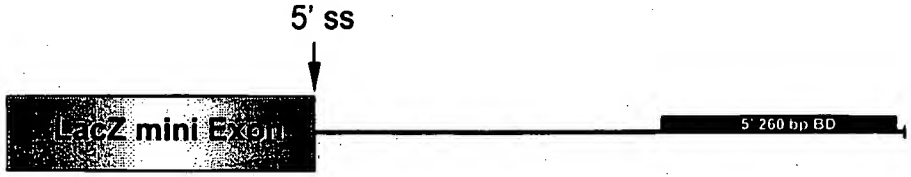
DSPTM8 :(▲ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)



Mutants

Figure 22

Accuracy of Double *Trans*-splicing Reaction

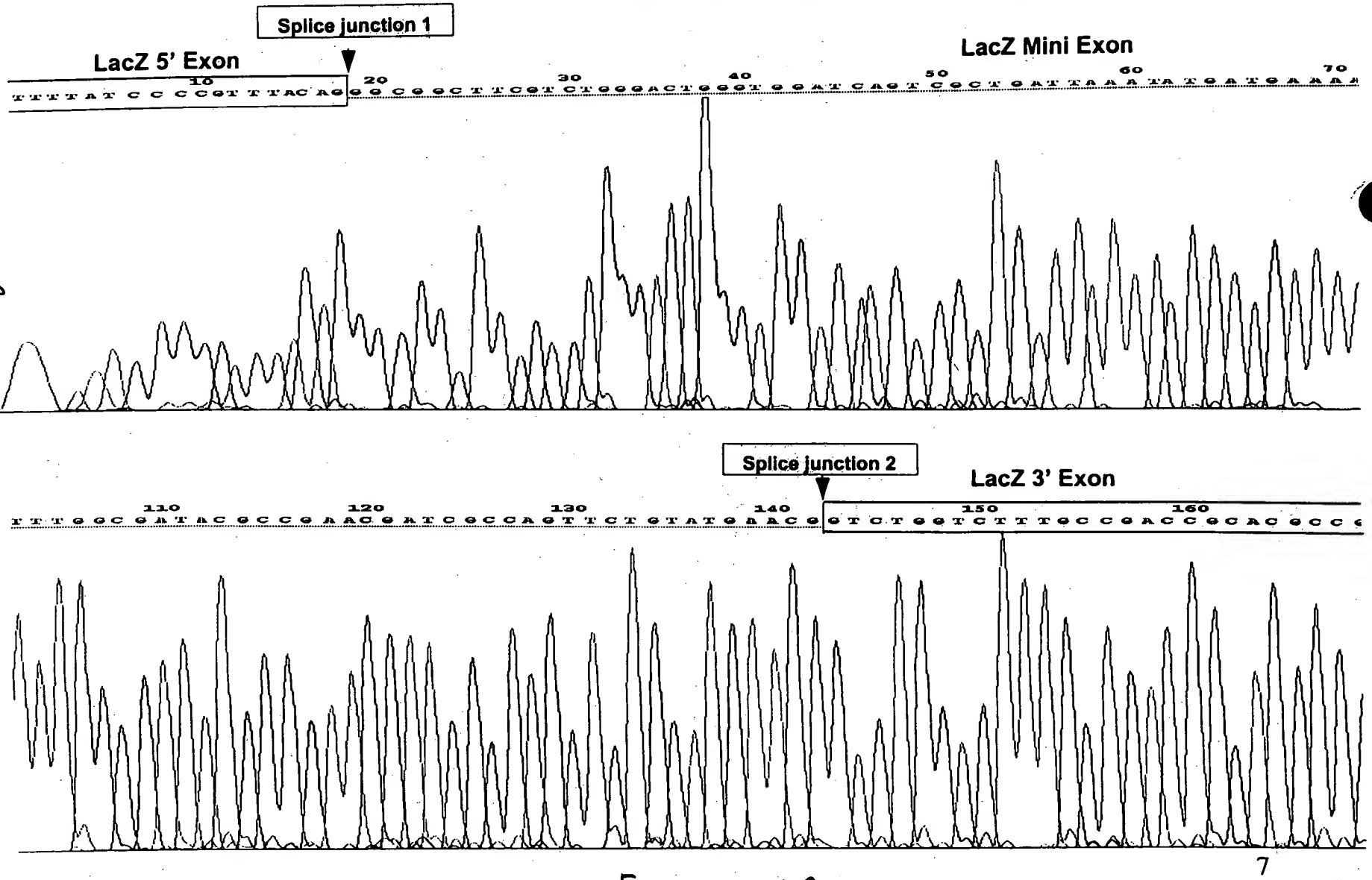
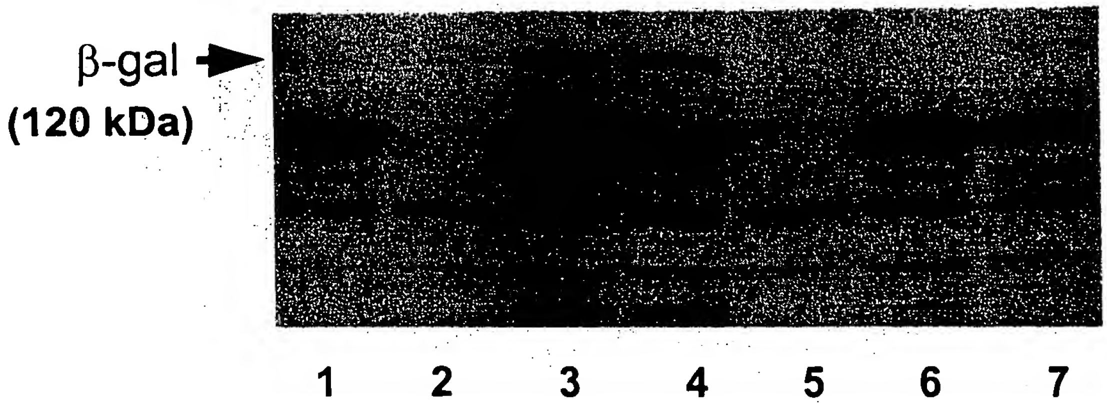


Figure 23

Double *Trans*-splicing Produces Full-length Protein

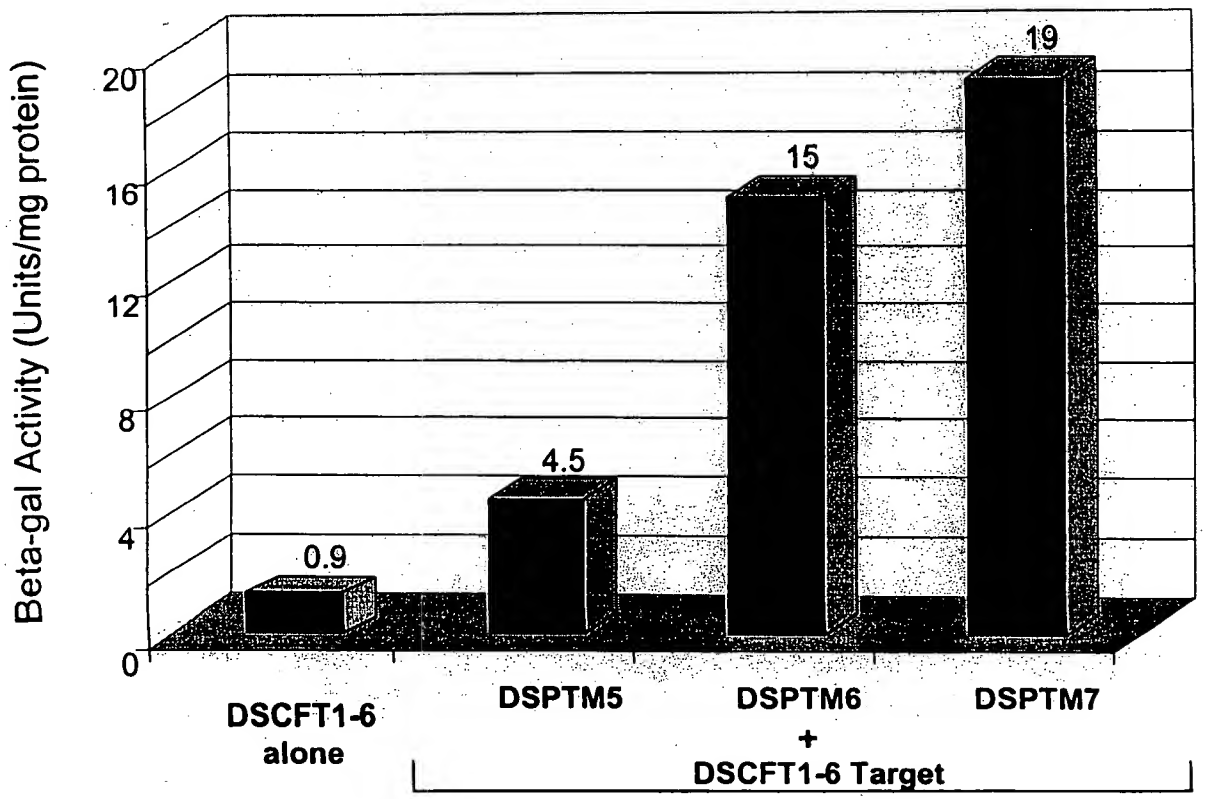


Lane 1: DSCFT1.6 Target alone	25 µg
Lane 2: DSPTM7	25 µg
Lane 3 Target + PTM #6	25 µg
Lane 4: Target + PTM #9	25 µg
Lane 5: Delta 3' splice mutant alone	25 µg
Lane 6: Target + Delta 3' ss	25 µg
Lane 7: Target+PTM29+30 (mutants)	25 µg

Figure 24

Sheet 31 of 58

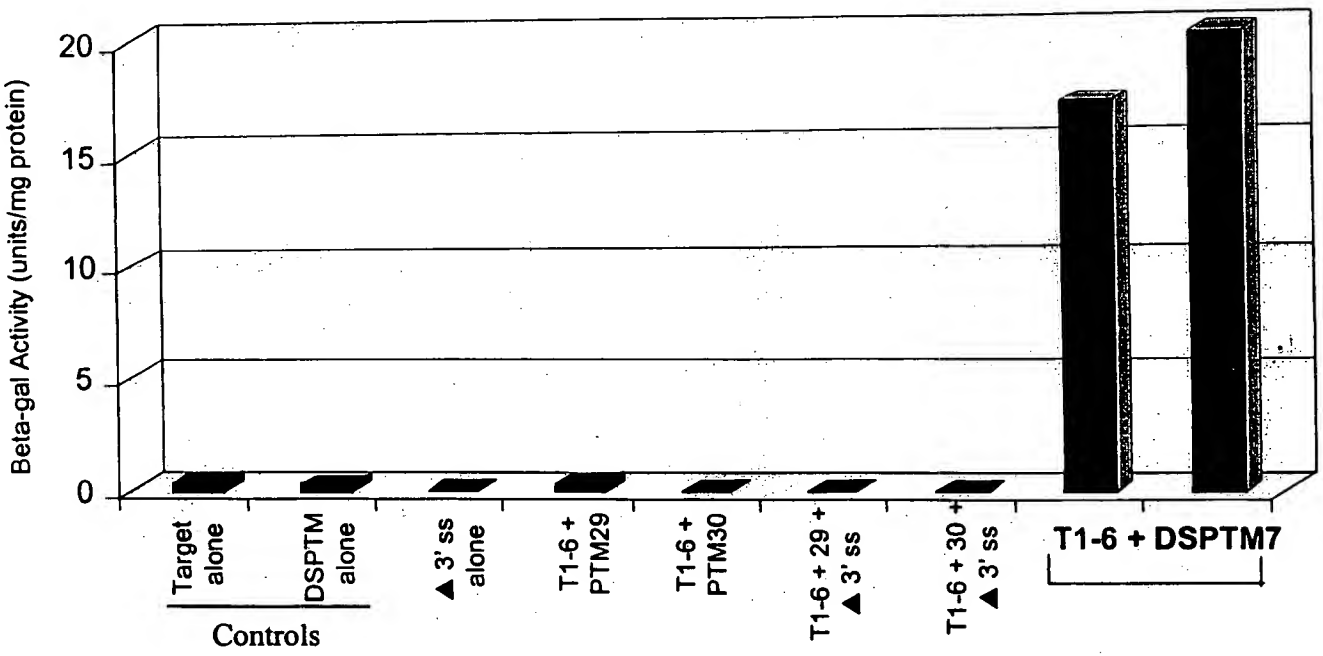
Restoration of β-Gal Function by Double *Trans*-splicing

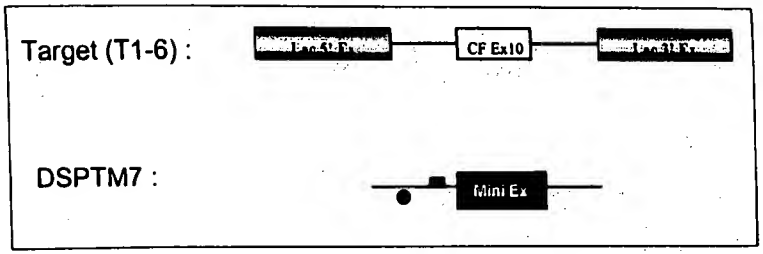


Beta-gal Activity above the Background level	
DSPTM5:	5 fold
DSPTM6:	17 fold
DSPTM7:	21 fold

Figure 25

Restoration of β-gal activity is due to double RNA *trans*-splicing events





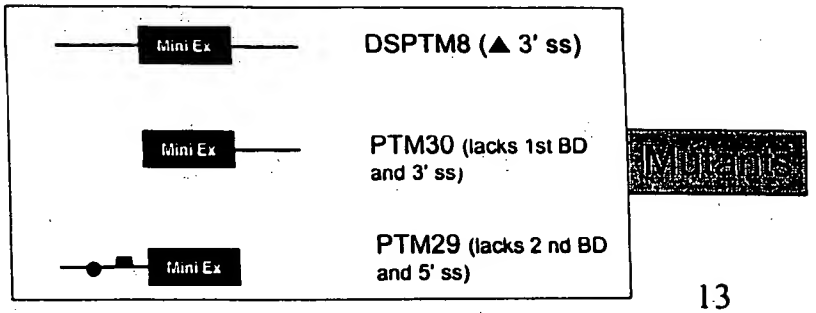
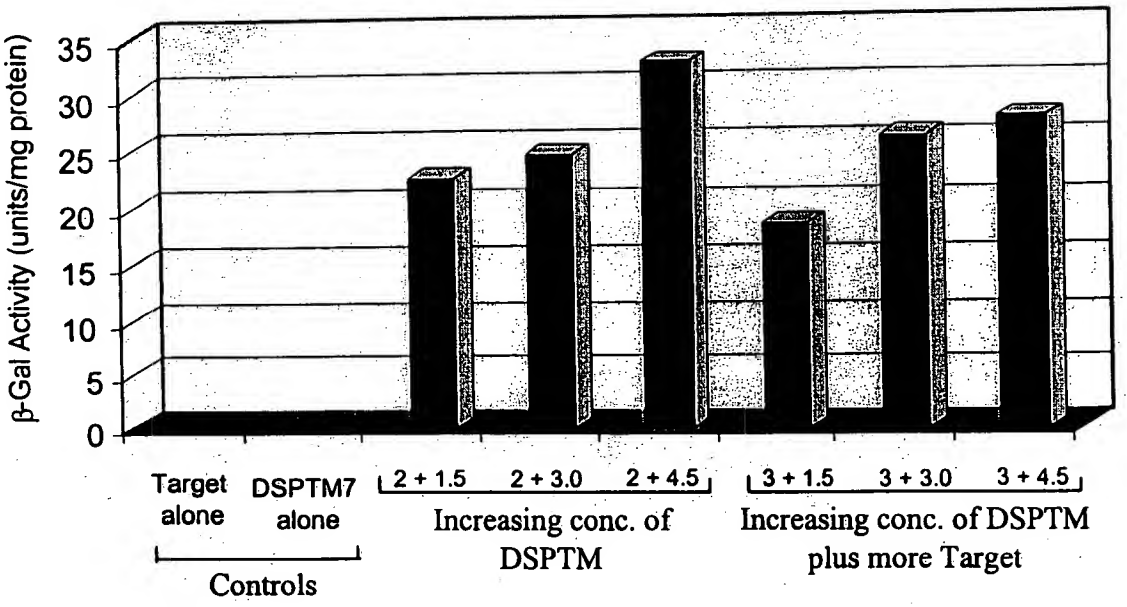


Figure 26

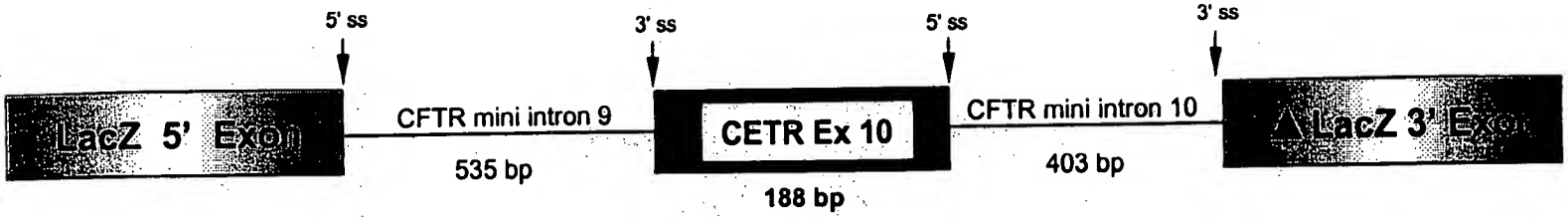
Double *Trans*-splicing: Titration of Target & PTM



The current level of beta-gal activity due to double *trans*-splicing is ~ 1-1.5% of the best single splice model (3' exon replacement)

Figure 27

DSCFT1-6 (Specific Target):



DSHCGT1 (Non-specific Target):

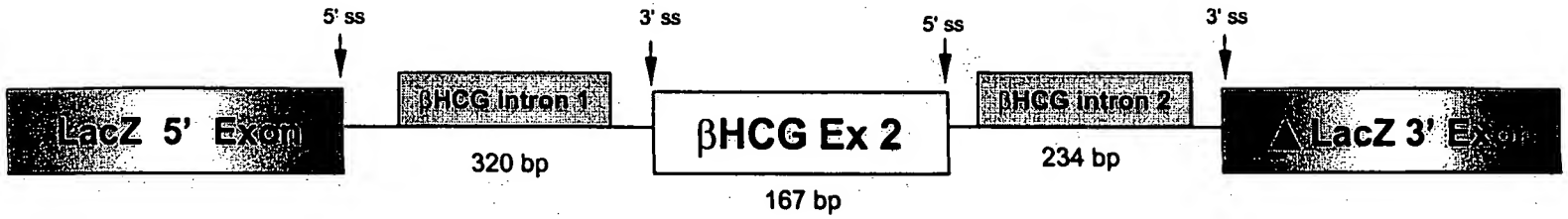


Figure 28

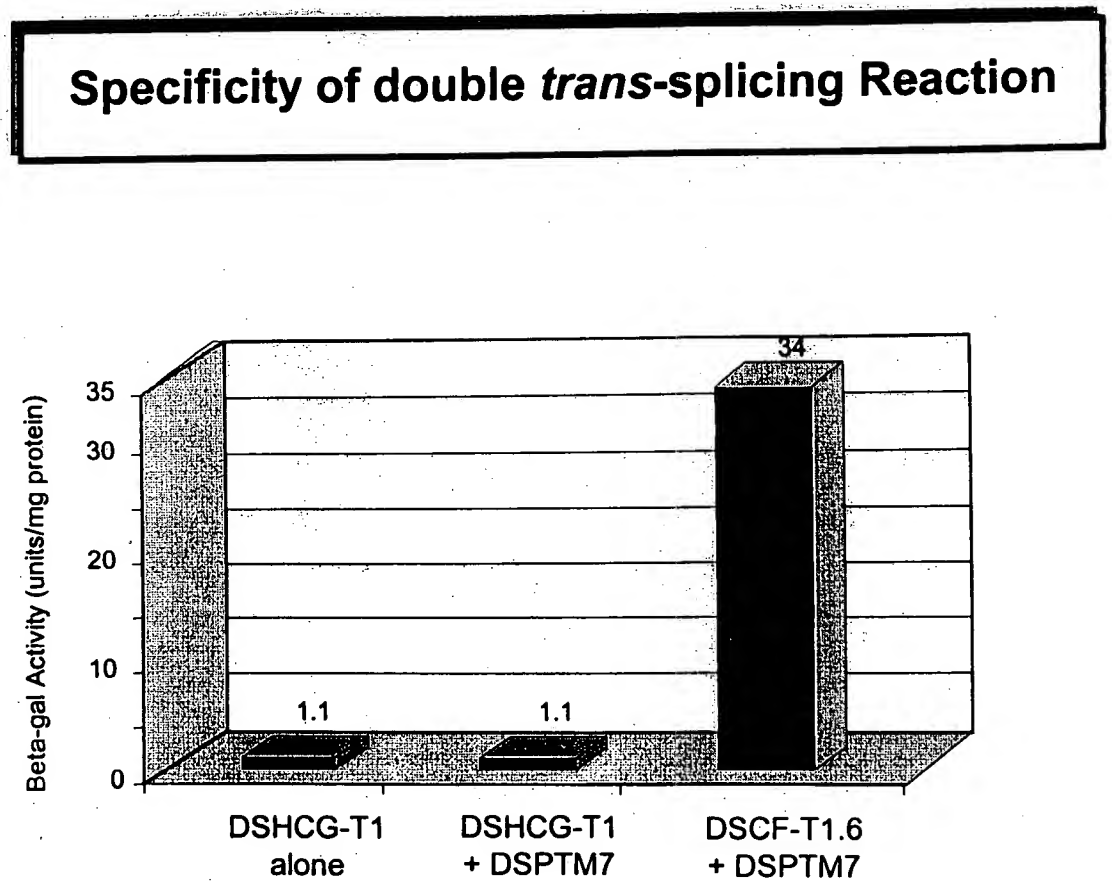
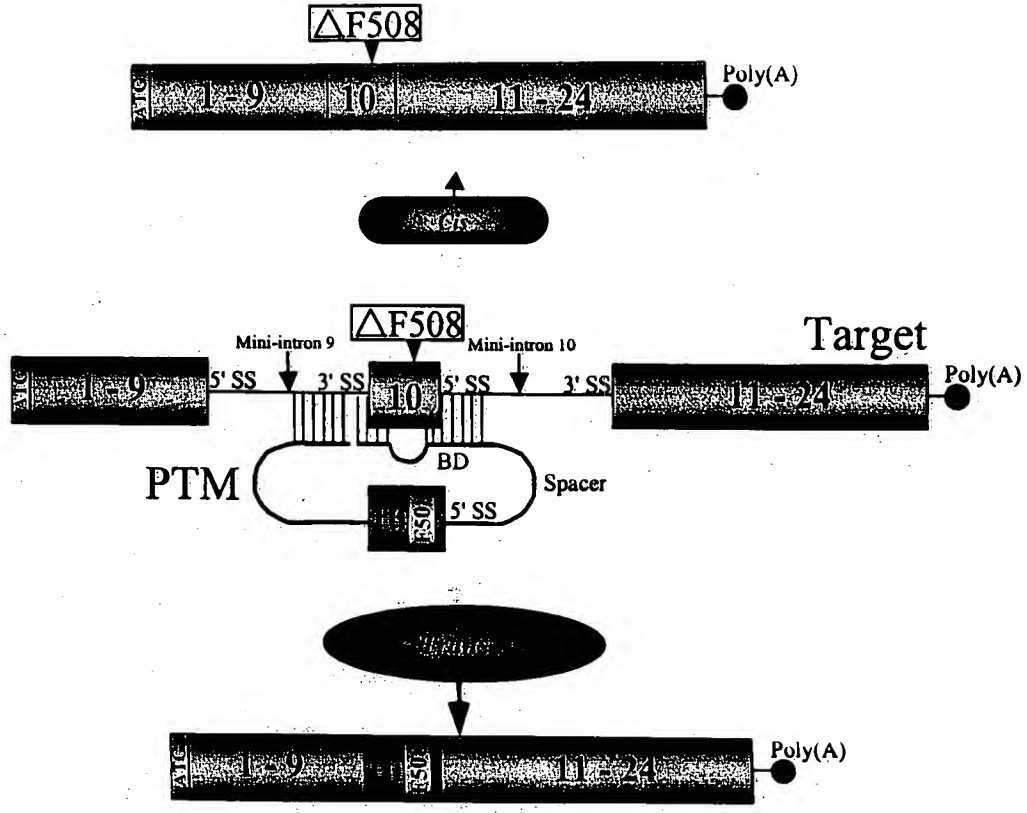

Specificity of double *trans*-splicing Reaction
Beta-gal Activity (units/mg protein)
35
30
20
10
0
1.1
1.1
34
DSHCG-T1 alone
DSHCG-T1 + DSPTM7
DSCF-T1.6 + DSPTM7

Figure 29

Replacement of a Single Internal Exon

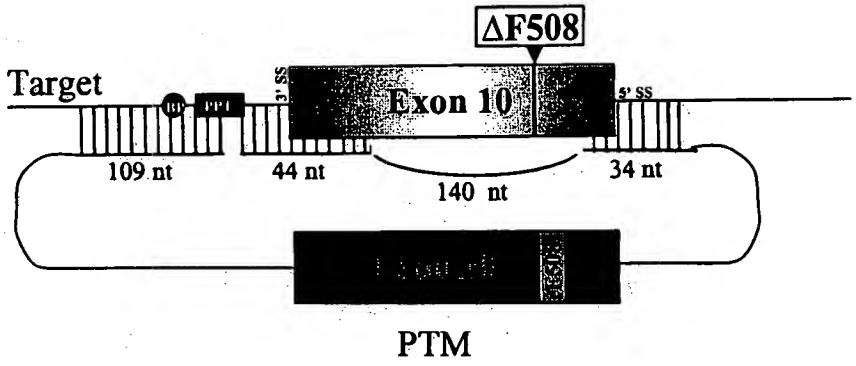
Schematic diagram of a PTM binding to a CFTR ΔF508 target



Repaired full length CFTR mRNA

Figure 30

PTM with a long binding domain masking two splice sites and part of exon 10 in a mini-gene target.



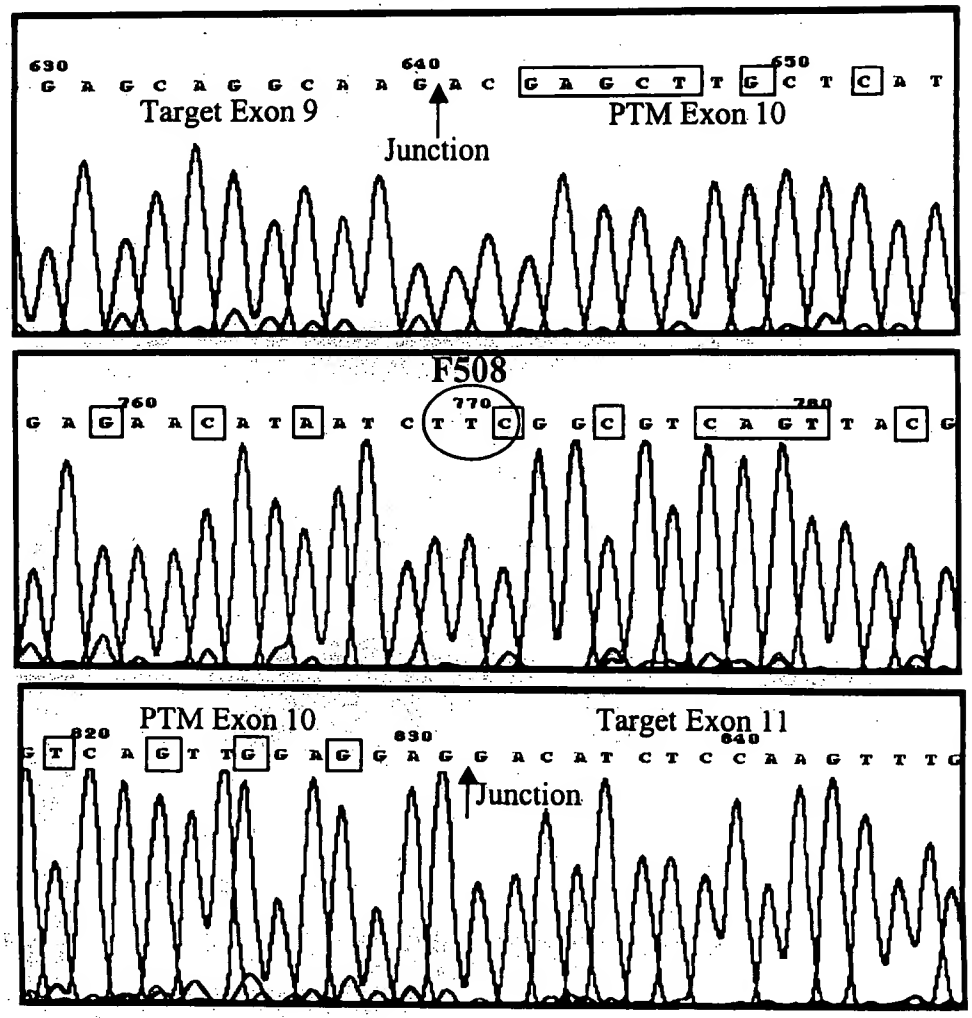
ACGAGCTTGCTCATGATGATCATGGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAACATTCCG
GCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAACATAAT
CTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCTGTCAGTTGGAGGAG

MCU in exon 10 of PTM

88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain (bold and underlined).

Figure 31

Sequence of a double *trans*-spliced product



☐ = MCU in PTM exon 10

Figure 32

CFTR Repair: 5' Exon Replacement

Schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

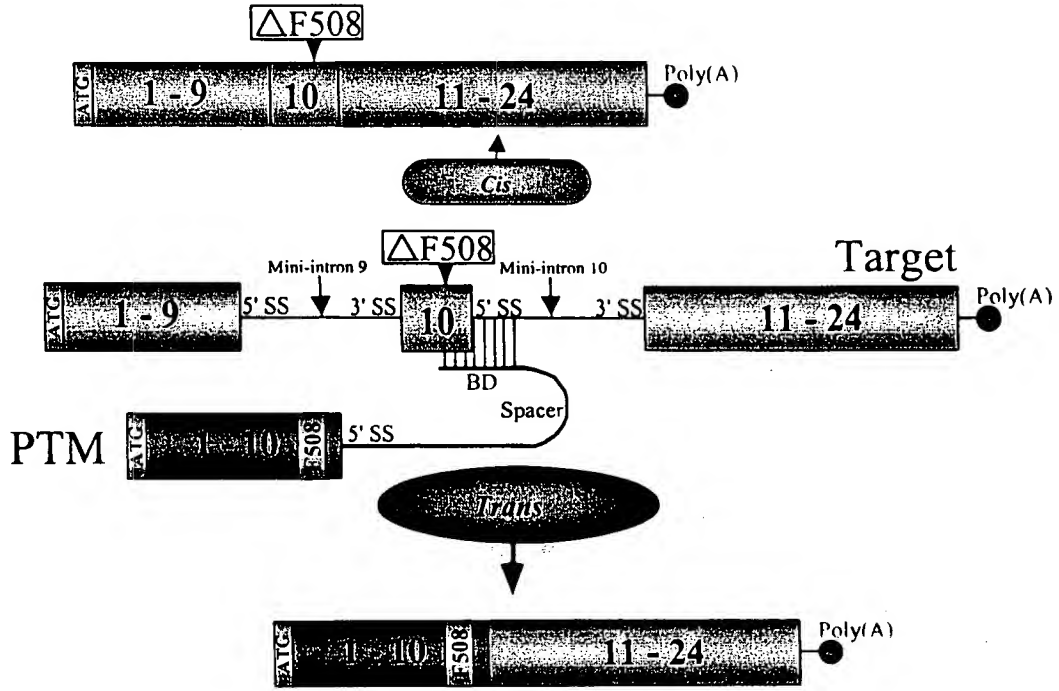
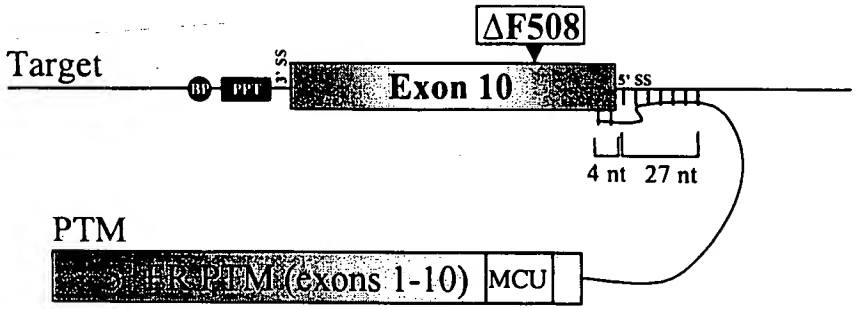


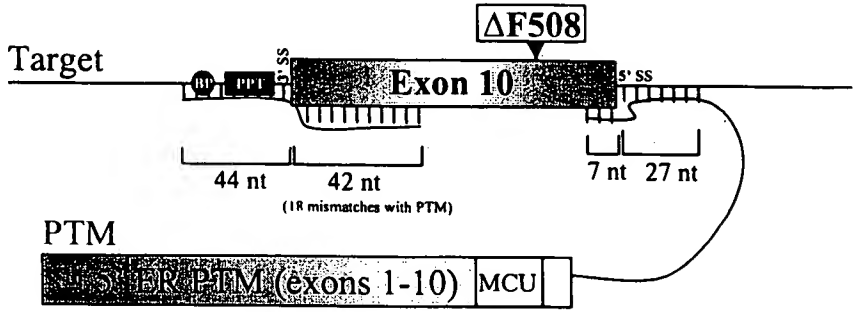
Figure 33

A



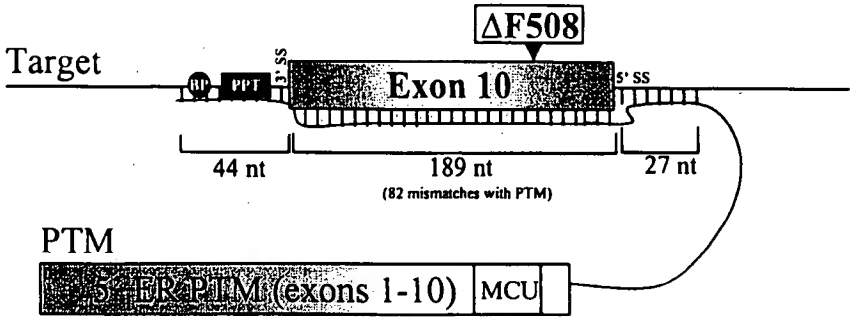
PTM with a short binding domain masking a single splice site in a mini-gene target.

B



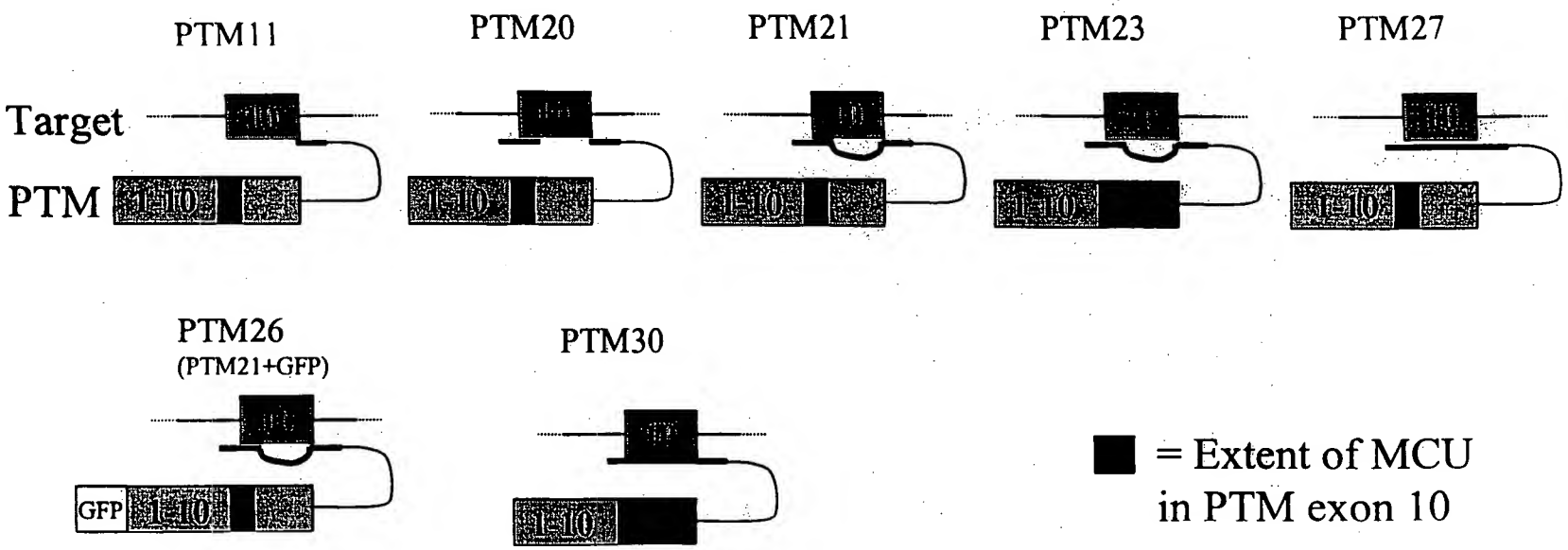
PTM with a long binding domain masking two splice sites in a mini-gene target.

C



PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

Figure 34

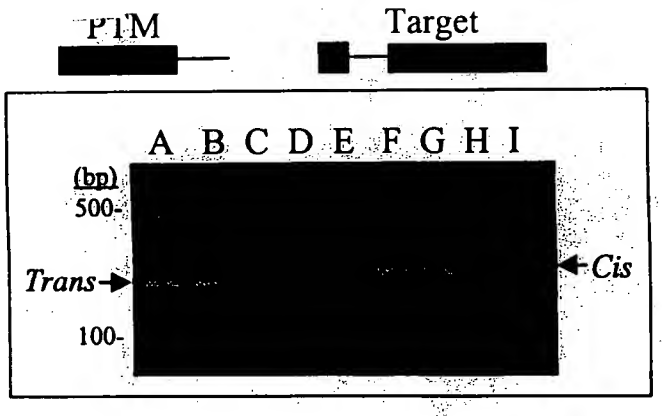


MCU in exon 10 of PTM

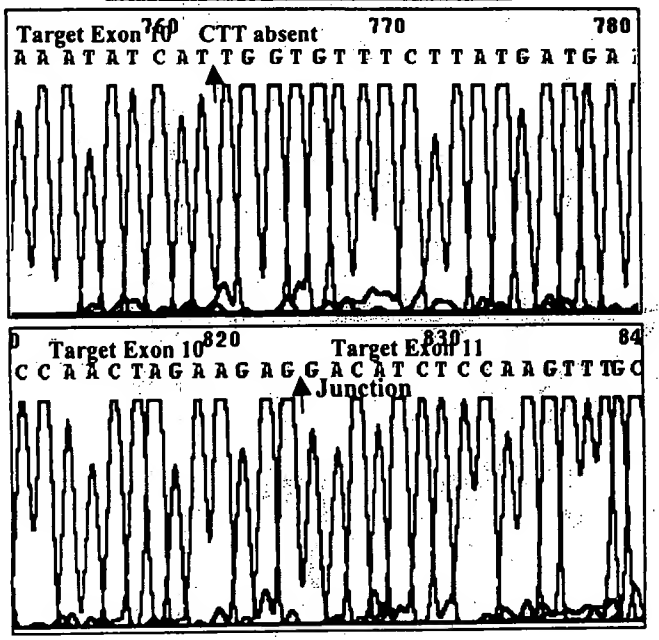
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGCTCATGATGATCATGGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAACATTCCG
GCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAACATAAT
CTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCTGTCAGTTGGAGGAG

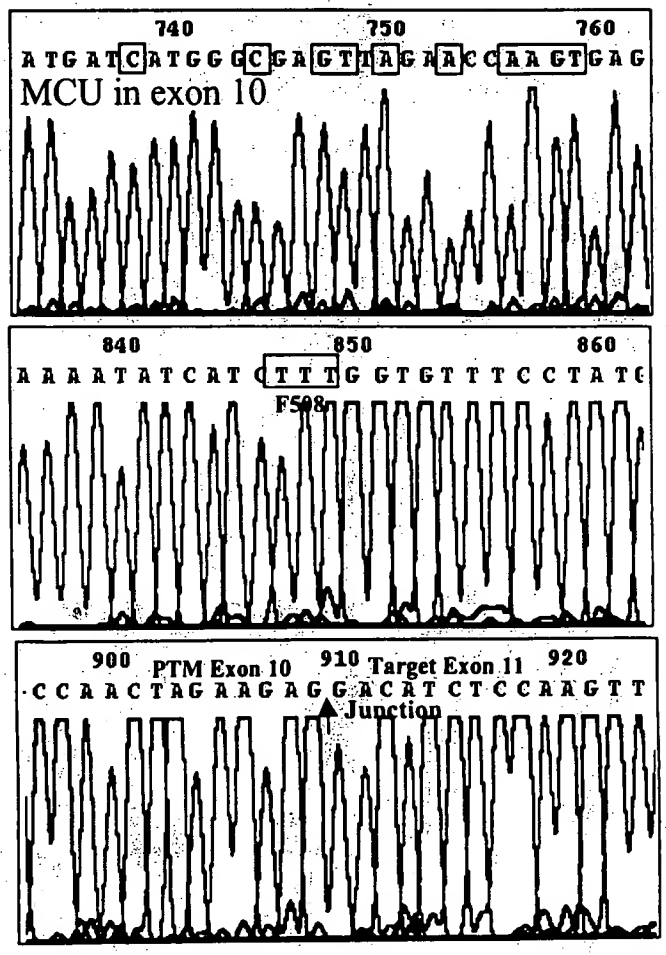
Figure 35



A. *Cis*-spliced product
[Primers CF1 + CF111]



B. *Trans*-spliced product
[Primers CF93 + CF111]



5

Figure 36

A

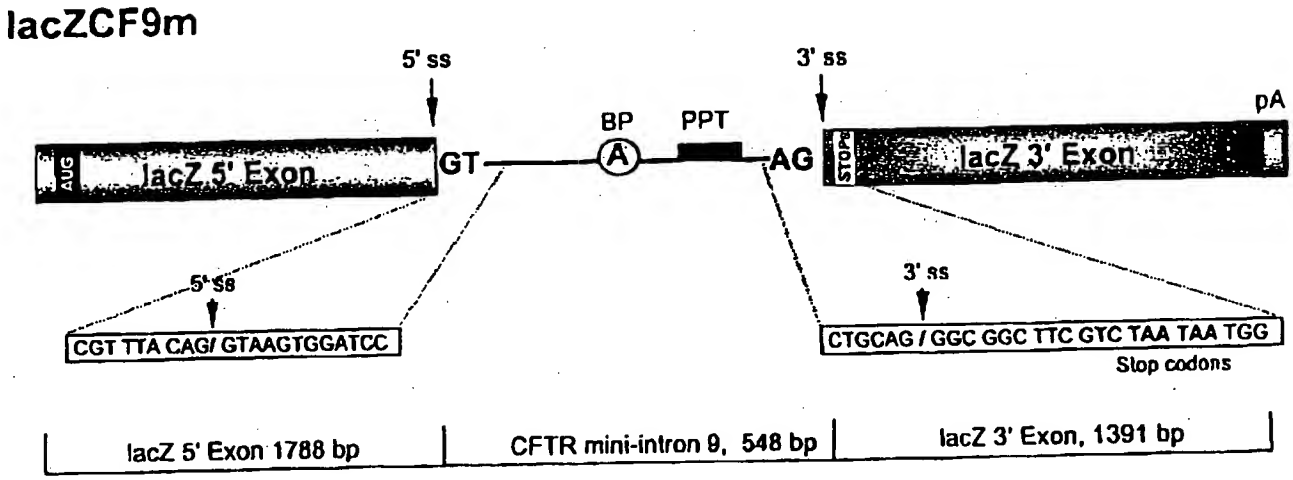


Figure 37 A

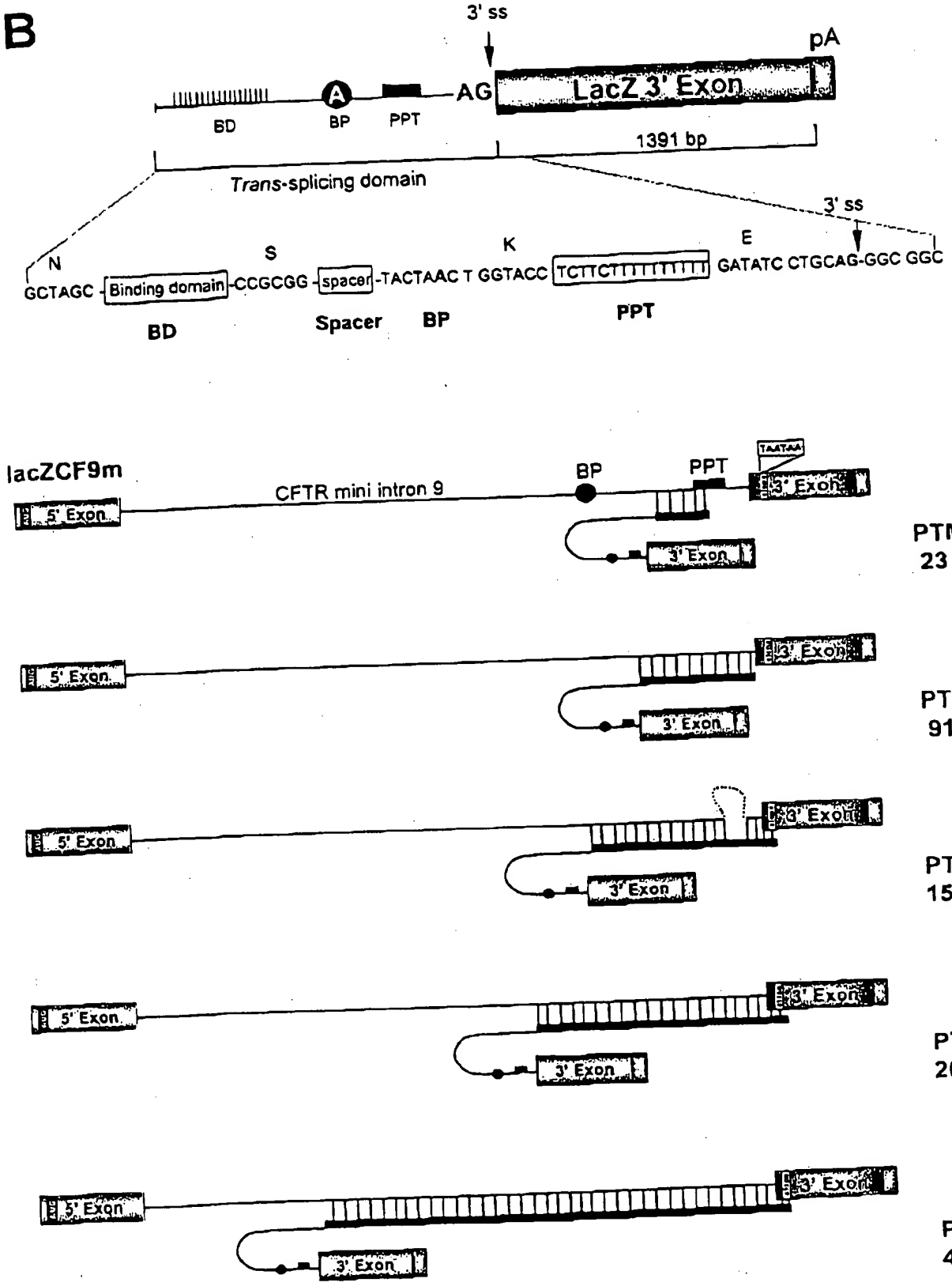

B
3' ss
pA
AG
LacZ 3' Exon
BD
BP
PPT
1391 bp
Trans-splicing domain
3' ss
N
S
K
E
GCTAGC - Binding domain - CCGCGG - spacer - TACTAAC T GGTACC TCTTCTTTTTTTTT GATATC CTGCAG-GGC GGC
BD
Spacer
BP
PPT
lacZCF9m
CFTR mini intron 9
BP
PPT
TAATAA
5' Exon
3' Exon
PTM-CF14
23 bp BD
PTM-CF22
91 bp BD
PTM-CF24
153 bp BD
PTM-CF26
200 bp BD
PTM-CF27
411 bp BD

Figure 37B

C

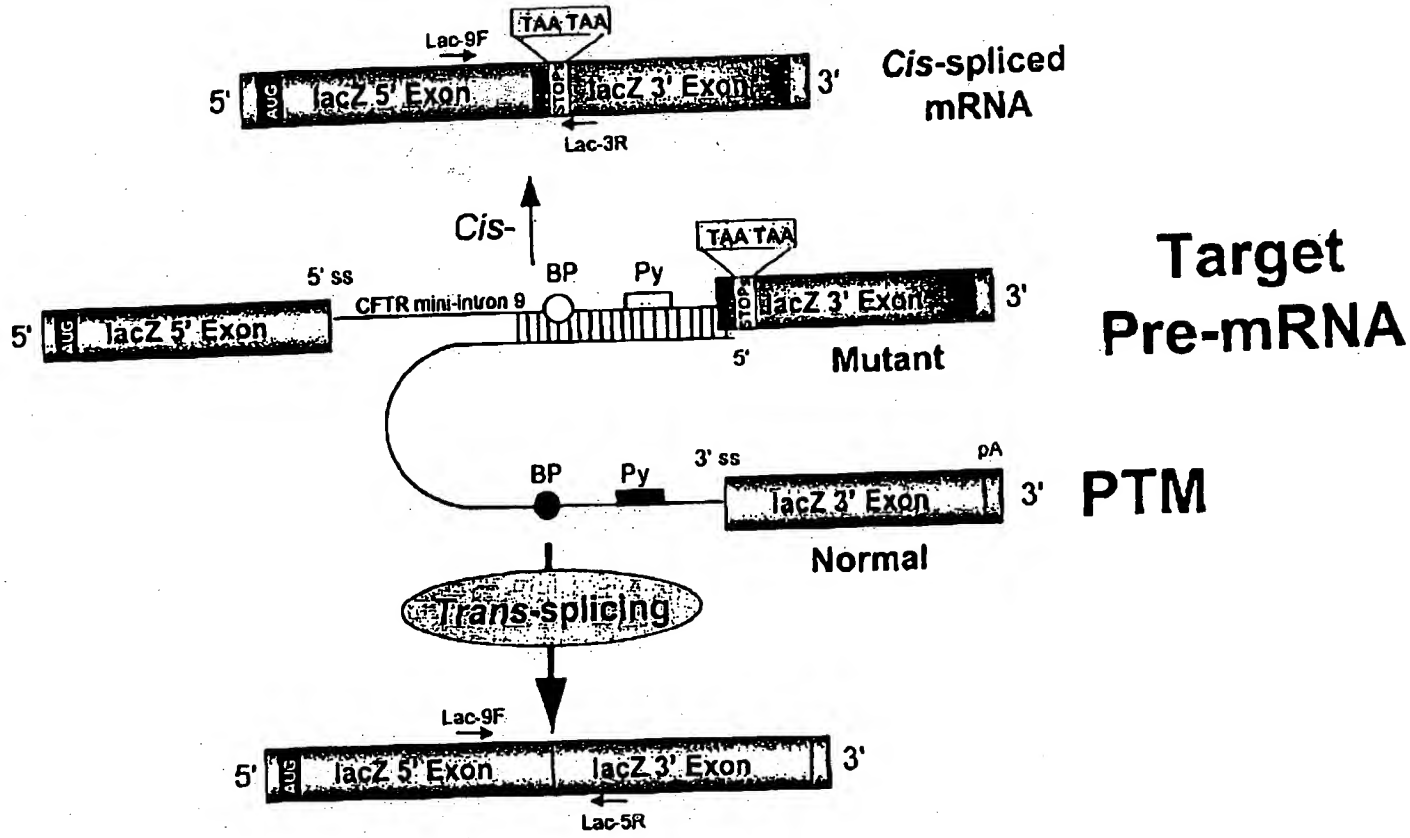

Lac-9F
TAA TAA
5'
AUG
lacZ 5' Exon
STOP
lacZ 3' Exon
3'
Lac-3R
Cis-spliced mRNA
Cis-
TAA TAA
5' ss
BP
Py
CFTR mini-intron 9
5'
AUG
lacZ 5' Exon
STOP
lacZ 3' Exon
3'
5'
Mutant
Target Pre-mRNA
BP
Py
3' ss
pA
lacZ 3' Exon
3'
Normal
PTM
Trans-splicing
Lac-9F
5'
AUG
lacZ 5' Exon
lacZ 3' Exon
3'
Lac-5R
Repaired lacZ mRNA

Figure 37C

A

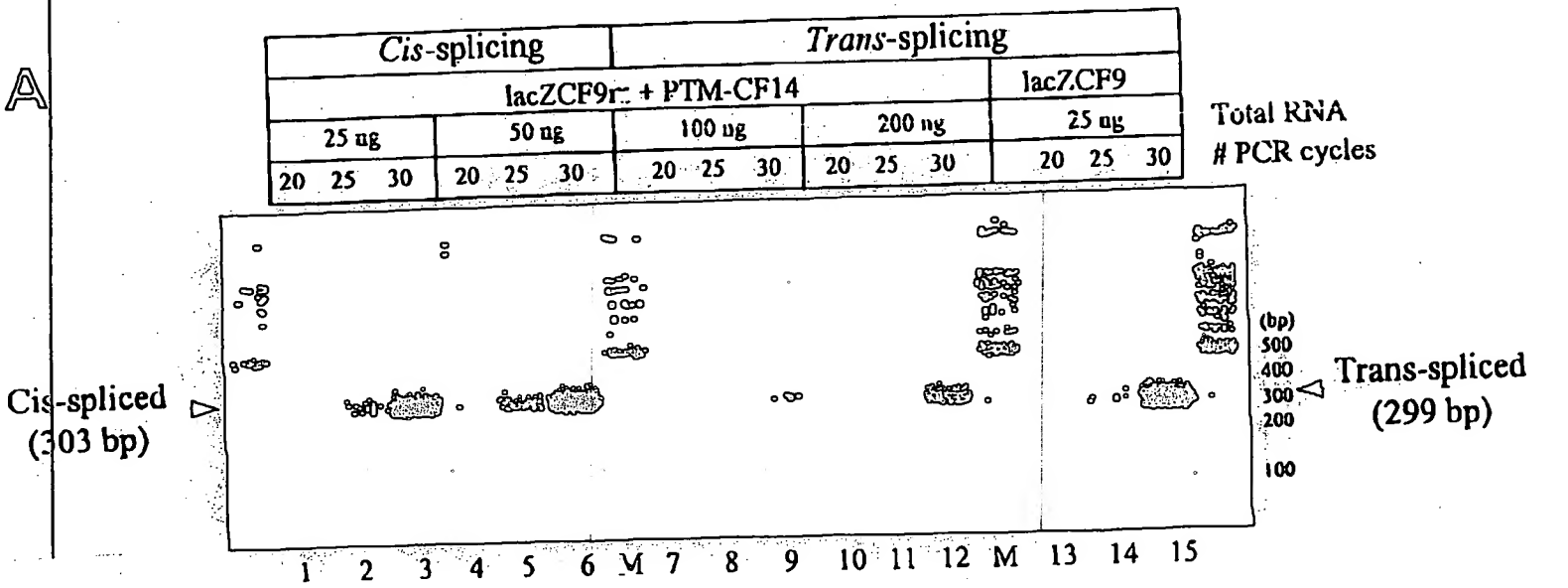
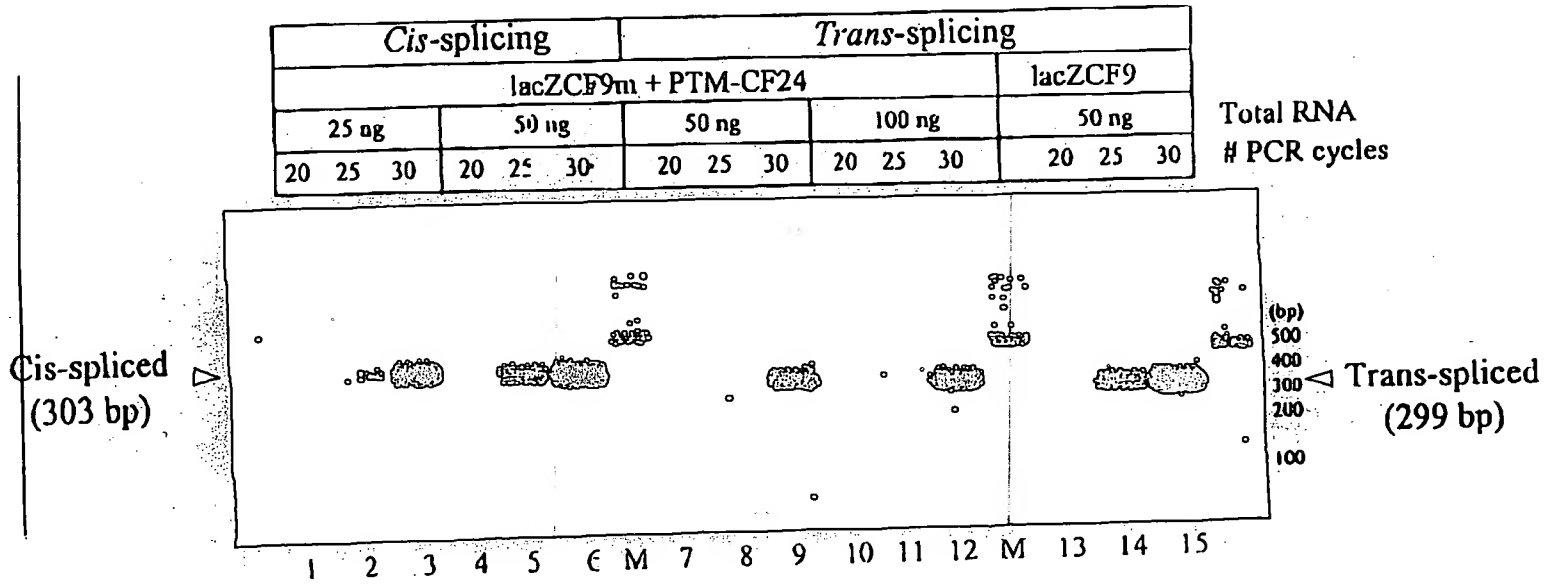

Cis-splicing
Trans-splicing
lacZCF9m + PTM-CF14
lacZCF9
25 ng
50 ng
100 ng
200 ng
25 ng
20 25 30
Total RNA
PCR cycles
Cis-spliced
(303 bp)
Trans-spliced
(299 bp)
(bp)
500
400
300
200
100
1 2 3 4 5 6 M 7 8 9 10 11 12 M 13 14 15

Figure 38 A


Cis-splicing
Trans-splicing
lacZCF9m + PTM-CF24
lacZCF9
25 ng
50 ng
50 ng
100 ng
50 ng
20 25 30
Total RNA
PCR cycles
Cis-spliced
(303 bp)
Trans-spliced
(299 bp)
(bp)
500
400
300
200
100
1 2 3 4 5 6 M 7 8 9 10 11 12 M 13 14 15

B

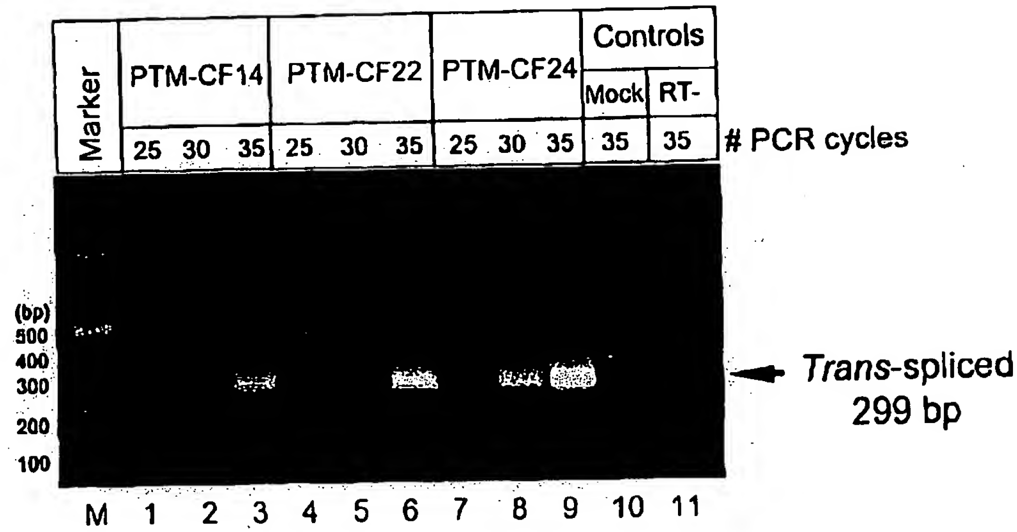

Marker
PTM-CF14
PTM-CF22
PTM-CF24
Controls
Mock
RT-
25 30 35 25 30 35 25 30 35 35 35
PCR cycles
(bp)
500
400
300
200
100
Trans-spliced
299 bp
M 1 2 3 4 5 6 7 8 9 10 11

Figure 38B

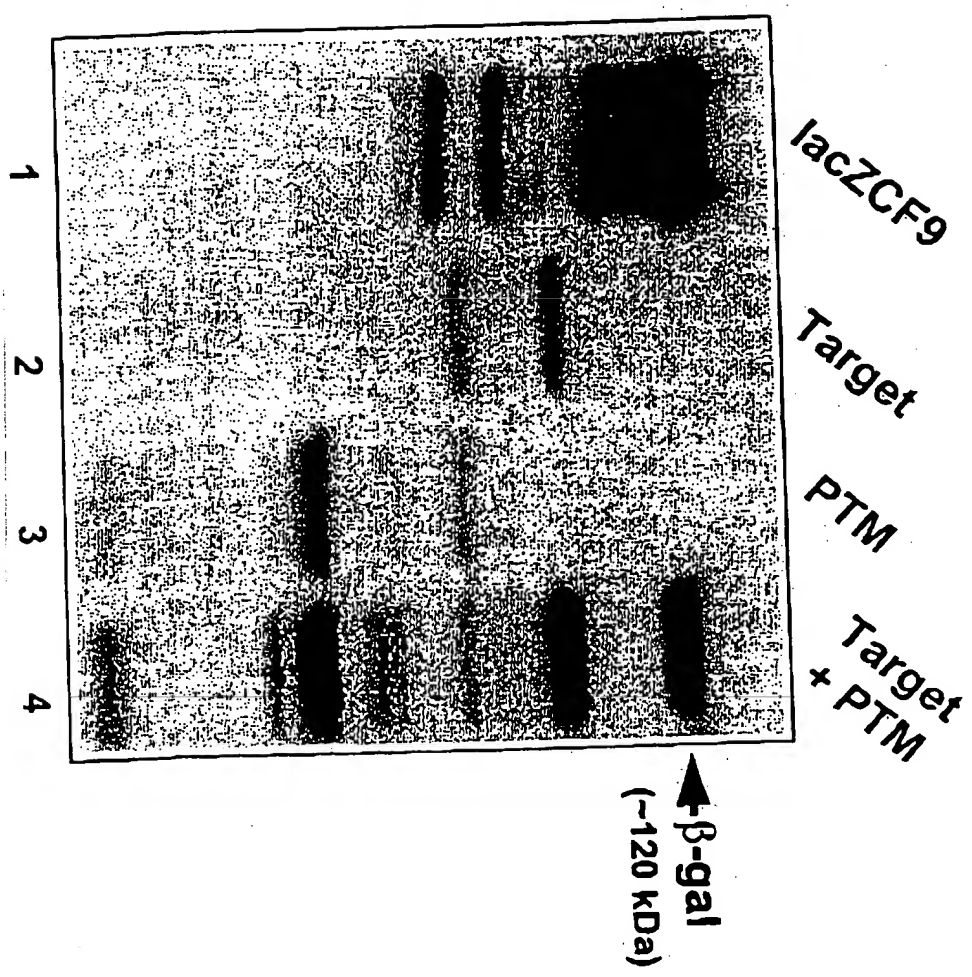


Figure 39

A

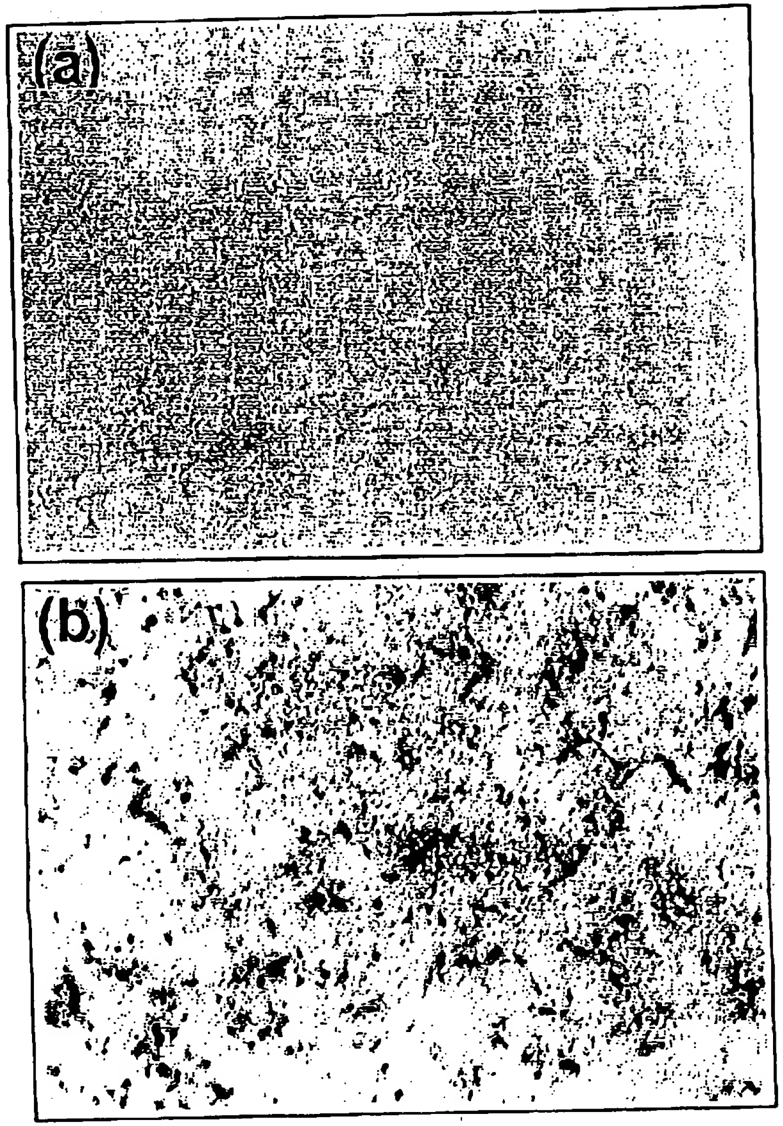


Figure 40A

Sheet 50 of 58

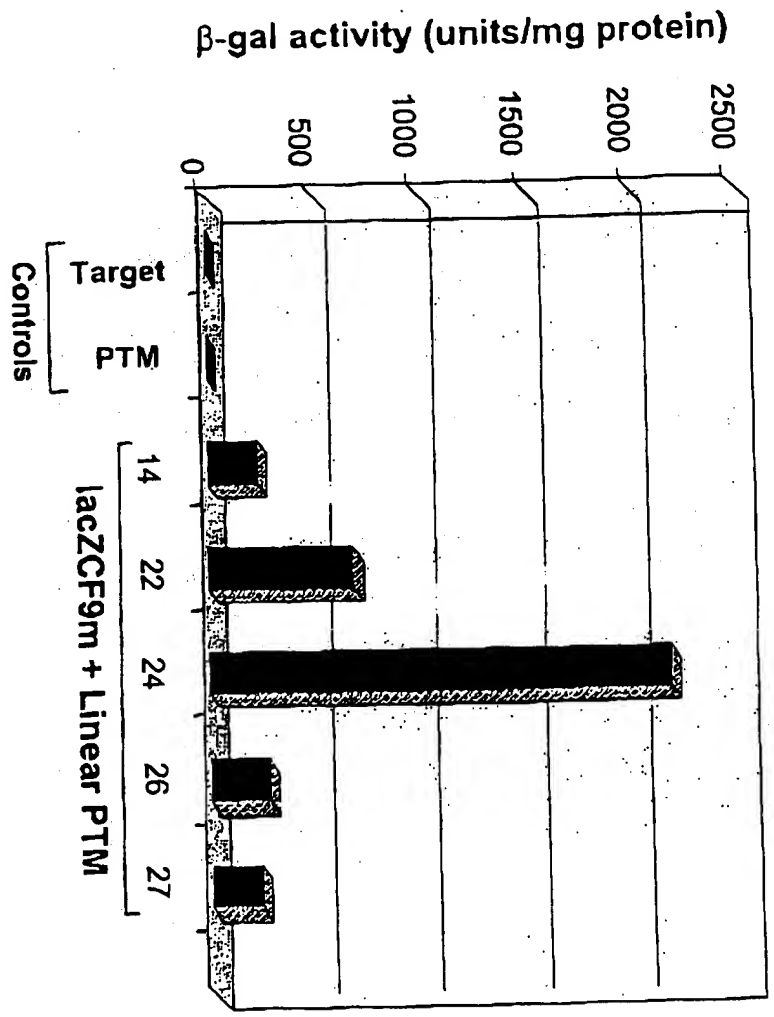

B
β-gal activity (units/mg protein)
0
500
1000
1500
2000
2500
Controls
Target
PTM
lacZCF9m + Linear PTM
14
22
24
26
27

Figure 40B

C

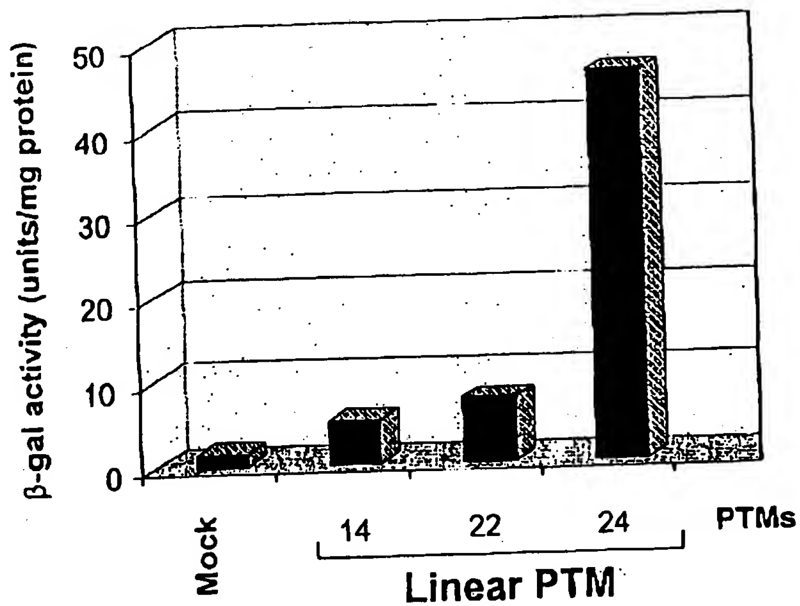

β-gal activity (units/mg protein)
0
10
20
30
40
50
Mock
14
22
24
PTMs
Linear PTM

Figure 40C

A

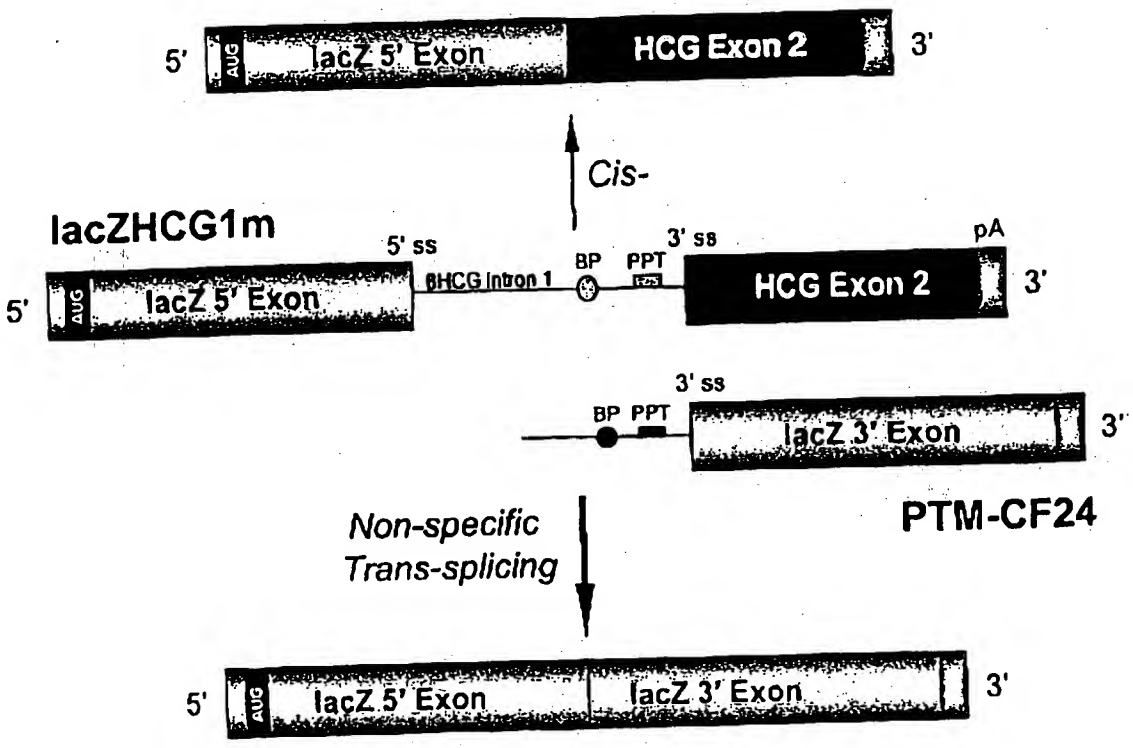

5'
AUG
lacZ 5' Exon
HCG Exon 2
3'
Cis-
lacZHCG1m
5' ss
3' ss
pA
BP
PPT
5'
AUG
lacZ 5' Exon
βHCG Intron 1
HCG Exon 2
3'
3' ss
BP PPT
lacZ 3' Exon
3'
PTM-CF24
Non-specific
Trans-splicing
5'
AUG
lacZ 5' Exon
lacZ 3' Exon
3'
Repaired lacZ mRNA

Figure 41A

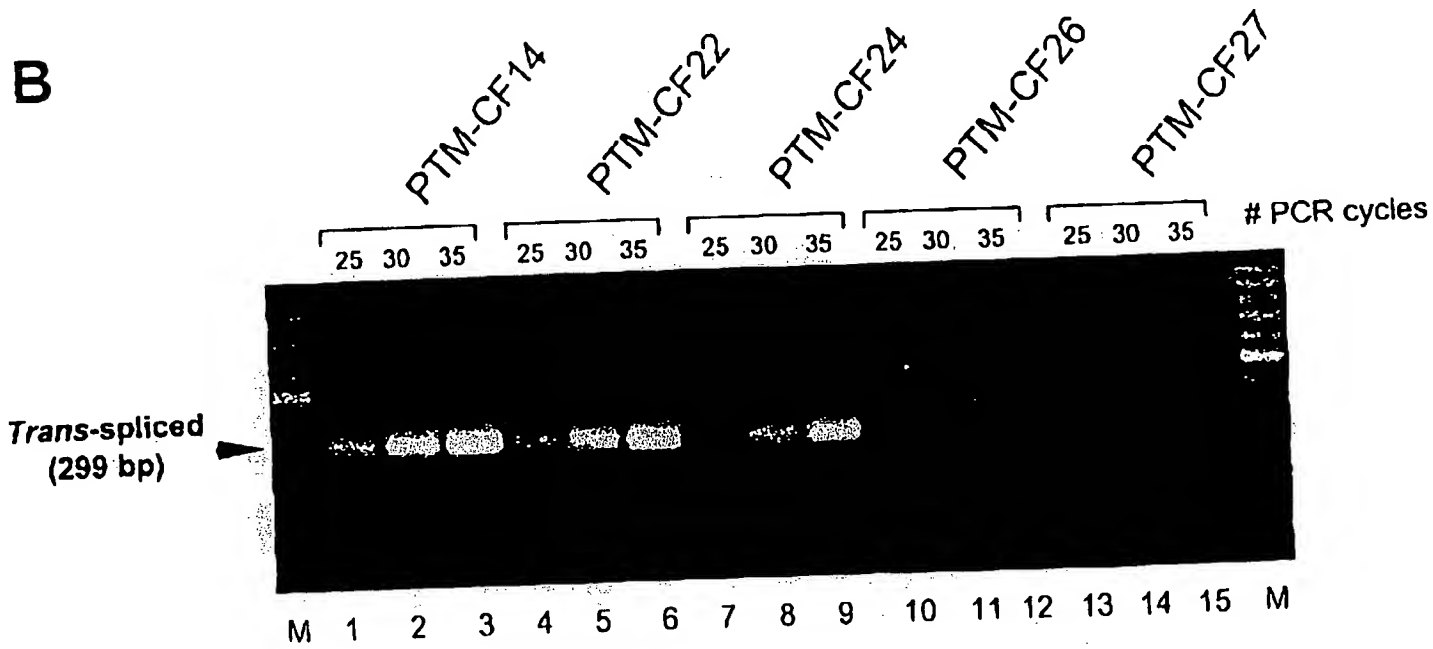


Figure 4B

C

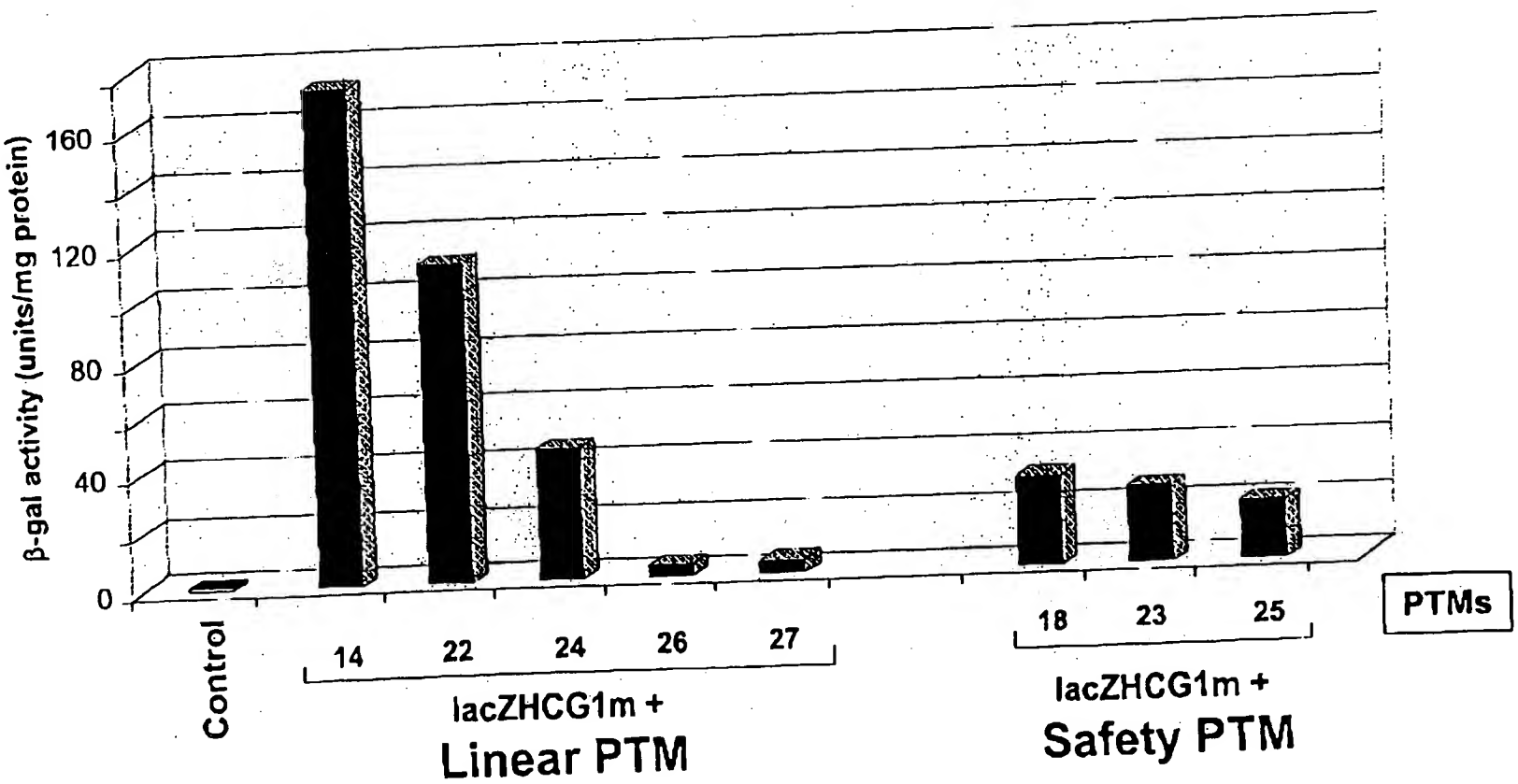

β-gal activity (units/mg protein)
160
120
80
40
0
Control
14
22
24
26
27
lacZHCG1m +
Linear PTM
18
23
25
PTMs
lacZHCG1m +
Safety PTM

Figure 41C

Exons 1-10

ATGCAGAGGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG
GATACAGACAGCGCCTGGAATTGTCAGACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT
GGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGATGTTTTTTCTGG
AGATTTATGTTCTATGGAATCTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA
TAGCTTCCTATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTAT
TGTGAGGACACTGCTCCTACACCCAGCCATTTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGT
TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACTTGTTAGTCTCCTTT
CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCACATTTCGTGTGGATCGCTCCTTTGCAAGTGGCACTCCT
CATGGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCCTGATAGTCCTTGCCCTTTTTCAG
GCTGGGCTAGGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG
AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGAAAAAATGATTGAAAACTTAAGACA
AACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTTCTTT
GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGGAAAATATTCACCACCATCTCATTCT
GCATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA
CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG
AATGTAACAGCCTTCTGGGAGGAGGGATTTGGGGAATTATTTGAGAAAGCAAAACAAAACAATAACAATAGAAAAACTT
CTAATGGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCTTGGTACTCCTGTCCTGAAAGATATTAATTTCAAGAT
AGAAAGAGGACAGTTGTTGGCGGTTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTGCTCATGATGATCATGGGCGAG
TTAGAACCAAGTGAAGGCAAGATCAAACATTCCGGCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTA
CCATCAAGGAGAACATAATCTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCTGTCAGTTGGA
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACGCTAAGATCCACCGG
TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG
GAAACACCAATGATATTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGT
GCTTAATTTTACCCTCTGAATTCTCCATTTCTCCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATT
ATTAACTCATTATCAAATCACGCT

Figure 42

153 bp PTM24 Binding Domain:

Nhe I 153 bp BD underlined

GCTAGC-AATAATGACGAAGCCGCCCCTCACGCTCAGGATTCACTTGCCTCCAATTATCATCCTAAGCAGAAGTGTATA

TTCTTATTTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAAATACTTCCTGTTTCACCTACTCTGCTATGC

Sac II

AC-**CCGCGG**

Sheet 57 of

Figure 43A

Trans-splicing domain
AATAATGACGAAGCCGCCCCTCACGCTCAGGATTCACTTGCCCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTA
TTTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAAATACTTCCTGTTTCACCTACTCTGCTATGCACCCGC
GGAACATTATTATAACGTTGCTCGAATACTAACTGGTACCTCTTCTTTTTTTTTTGATATCCTGCAG
Exons 10-24
ACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGGAAGAATTTCATTCT
GTTCTCAGTTTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTTCCTATGATGAATATAGATA
CAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTTCTTGGAGAA
GGTGGAATCACACTGAGTGGAGGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTTGTATT
TATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAGAAATATTTGAAAGCTGTGTCTGTAAACTGATGGC
TAACAAAACTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCATGAAGGT
AGCAGCTATTTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAAACTCATGGGATGTGATT
CTTTCGACCAATTTAGTGCAGAAAGAAGAAATTCAATCCTAACTGAGACCTTACACCGTTTCTCATTAGAAGGAGATGC
TCCTGTCTCCTGGACAGAAACAAAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAAAGGAAGAATTCTATT
CTCAATCCAATCAACTCTATACGAAAATTTTCCATTGTGCAAAAGACTCCCTTACAAATGAATGGCATCGAAGAGGATT
CTGATGAGCCTTTAGAGAGAAGGCTGTCCTTAGTACCAGATTCTGAGCAGGGAGAGGCGATACTGCCTCGCATCAGCGT
GATCAGCACTGGCCCCACGCTTCAGGCACGAAGGAGGCAGTCTGTCCTGAACCTGATGACACACTCAGTTAACCAAGGT
CAGAACATTCACCGAAAGACAACAGCATCCACACGAAAAGTGTCACTGGCCCCTCAGGCAAACTTGACTGAACTGGATA
TATATTCAAGAAGGTTATCTCAAGAAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT
TTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCACAAGAGCTTA
ATTTTTGTGCTAATTTGGTGCTTAGTAATTTTTCTGGCAGAGGTGGCTGCTTCTTTGGTTGTGCTGTGGCTCCTTGGAA
ACACTCCTCTTCAAGACAAAGGGAATAGTACTCATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTC
GTATTATGTGTTTTACATTTACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATTCTTCAGAGGTCTACCACTGGTG
CATACTCTAATCACAGTGTCGAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCCTCA
ACACGTTGAAAGCAGGTGGGATTCTTAATAGATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGCCTCTTACCAT
ATTTGACTTCATCCAGTTGTTATTAATTGTGATTGGAGCTATAGCAGTTGTCGCAGTTTTACAACCCTACATCTTTGTT
GCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCCTCCAAACCTCACAGCAACTCAAACAACTGG
AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTTACAAGCTTAAAAGGACTATGGACACTTCGTGCCTTCGGACG
GCAGCCTTACTTTGAAACTCTGTTCCACAAAGCTCTGAATTTACATACTGCCAACTGGTTCTTGTACCTGTCAACACTG
CGCTGGTTCCAAATGAGAATAGAAATGATTTTTGTCATCTTCTTCATTGCTGTTACCTTCATTTCCATTTTAACAACAG
GAGAAGGAGAAGGAAGAGTTGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGGGCTGTAAACTC
CAGCATAGATGTGGATAGCTTGATGCGATCTGTGAGCCGAGTCTTTAAGTTCATTGACATGCCAACAGAAGGTAAACCT
ACCAAGTCAACCAAACCATACAAGAATGGCCAACTCTCGAAAGTTATGATTATTGAGAATTCACACGTGAAGAAAGATG
ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAAATGCCATATTAGA
GAACATTTCCTTCTCAATAAGTCCTGGCCAGAGGGTGGGCCTCTTGGGAAGAACTGGATCAGGGAAGAGTACTTTGTTA
TCAGCTTTTTTGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTGTCTTGGGATTCAATAACTTTGCAAC
AGTGGAGGAAAGCCTTTGGAGTGATACCACAGAAAGTATTTATTTTTTCTGGAACATTTAGAAAAAACTTGGATCCCTA
TGAACAGTGGAGTGATCAAGAAATATGGAAAGTTGCAGATGAGGTTGGGCTCAGATCTGTGATAGAACAGTTTCCTGGG
AAGCTTGACTTTGTCCTTGTGGATGGGGGCTGTGTCCTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG
TTCTCAGTAAGGCGAAGATCTTGCTGCTTGATGAACCCAGTGCTCATTTGGATCCAGTAACATACCAAATAATTAGAAG
AACTCTAAAACAAGCATTTGCTGATTGCACAGTAATTCTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA
TTTTTGGTCATAGAAGAGAACAAAGTGCGGCAGTACGATTCCATCCAGAAACTGCTGAACGAGAGGAGCCTCTTCCGGC
AAGCCATCAGCCCCTCCGACAGGGTGAAGCTCTTTCCCCACCGGAACTCAAGCAAGTGCAAGTCTAAGCCCCAGATTGC
Histidine tag Stop
TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATTAG

Figure 43B